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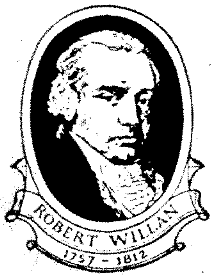

British Journal of Dermatology

CUK-H06077-32-P25446

Published for the
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DESPATCH. The Journal is despatched within Europe by surface mail, to other continents by various forms of air-speeded delivery: to the U.S.A.* by air freight for forwarding by second-class post, to India by air freight for guaranteed local delivery, and to all other countries by Accelerated Surface Post.

* Second-class postage paid at Rahway, NJ. Post Master, send address changes to *British Journal of Dermatology*, c/o Mercury Airfreight International Inc., 2323 Randolph Avenue, Avenel, NJ 07001, U.S.A.

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This journal is printed on acid-free paper.

P, 25,446

Printed in Great Britain at the Alden Press, Oxford

Vol. 125

Acc 10 1/73

British Journal of Dermatology
Volume 125, Number 1, July 1991

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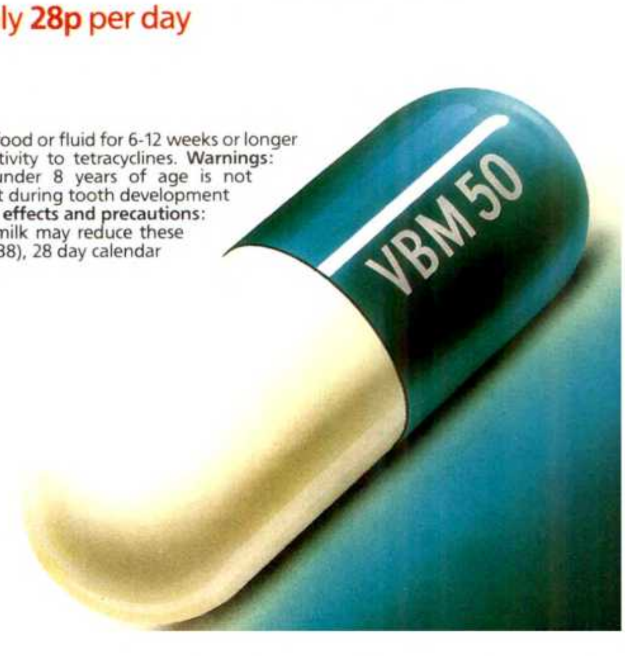

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Gamma glutamyl transpeptidase expression in foetal skin, inflammatory dermatoses and cutaneous neoplasia

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Accepted for publication 7 January 1991

Summary

Gamma glutamyl transpeptidase (GGT) is an enzyme expressed by some epithelial neoplasms but not normal interfollicular epidermis. In order to examine the relationship between malignant change and de-differentiation we studied histochemically the expression of GGT in human foetal skin, various inflammatory dermatoses and epidermal neoplasms. In foetal skin GGT was detectable after 7 weeks' gestation, reached a maximum at 11 weeks and was undetectable by 24 weeks. It was expressed strongly by squamous cell carcinoma and focally in Bowen's disease and actinic keratoses. There was no GGT expression in basal cell carcinoma or most benign skin tumours, but keratoacanthomas were weakly positive. Keratinocytes in the vicinity of malignant melanocytes also expressed GGT. This study suggests that GGT expression, while not a simple marker of malignancy, may represent reversion to a less differentiated or 'foetal' phenotype.

Gamma glutamyl transpeptidase (GGT) is a membrane-bound enzyme[1,2] present in a variety of normal mammalian tissues.[3,4] Its precise physiological role is uncertain, but it has been suggested that the enzyme plays a part in amino-acid transport[5] and glutathione catabolism.[6] Very high levels of the enzyme have been observed in certain malignant tumours including carcinomas of the colon[7] and lung,[8] and GGT has been shown to appear in the preneoplastic phases of experimental carcinogenesis in rat liver.[9] Serum levels of the enzyme are a sensitive indicator of malignant hepatic infiltration.[10,11]

In the skin, as with other tissues, aberrant expression of GGT seems closely linked to the development of cell transformation. The enzyme GGT was shown to be expressed by malignant keratinocytes in chemically induced murine squamous cell carcinomas.[12] More recently it has been shown that GGT expression correlates with both aneuploidy and loss of high MW differentiation-associated keratins in a two-stage animal carcinogenesis model.[13,14] Initial histochemical studies have suggested that GGT expression may be of use in distinguishing benign from malignant epidermal tumours in man.[15] Although once thought to be a marker of cellular proliferation, GGT expression is now better regarded as a marker of de-differentiation or regression to a foetal phenotype.[16] In support of this, GGT has been shown to be expressed in foetal rat skin,[17] but to date this has not been confirmed in man.

We have therefore studied the expression of GGT during human foetal skin development and in normal adult human skin. To reassess the use of GGT as a marker of malignancy we have examined its expression in benign, premalignant and malignant skin tumours, and have also looked at various inflammatory dermatoses.

Methods

Adult normal skin

Following informed consent, elliptical biopsies of normal skin were obtained under local anaesthetic from 32 patients undergoing minor surgery for other reasons. Patients ranged in age from 7 to 72 years.

Foetal skin

Foetal skin was obtained from different areas of the integument of 26 foetuses (gestation 5–24 weeks) legally aborted by suction. Gestational age was determined by the measurement of foot length.[18] Foetal skin samples were embedded in OCT compound, frozen in liquid nitrogen and stored at −70°C until used.

Correspondence: Dr D.M.MacDonald, Department of Dermatology, Guy's Hospital, St Thomas' Street, London SE1 9RT, U.K.

Table 1. Tumours and dermatoses studied

	Total	GGT
Malignant lesions		
Basal cell carcinoma	14	–
Squamous cell carcinoma	8	+
Bowen's disease	7	+
Actinic keratosis	6	+
Melanoma	6	Tumour –, Keratinocytes +
Paget's disease	2	Paget cells +, Keratinocytes –
Benign lesions		
Keratoacanthoma	3	+
Viral wart	4	–
Seborrhoeic keratosis	3	–
Trichoepithelioma	1	–
Melanocytic naevus	7	–
Histiocytoma	2	–
Keloid	2	–
Inflammatory dermatoses		
Flegel's disease	4	–
Psoriasis	4	–
Atopic dermatitis	3	–
Urticaria pigmentosa	3	Mast cells +, Keratinocytes –

Adult cutaneous lesions

A variety of benign and malignant cutaneous tumours and inflammatory dermatoses (Table 1) were subjected to elliptical biopsy under local anaesthesia. A portion of each lesion was processed for routine histological examination and stained with haematoxylin and eosin for diagnostic confirmation. The remainder was embedded in OCT compound, frozen and stored in liquid nitrogen.

Histochemical detection of GGT

Cryostat sections (6μm) were allowed to dry at room temperature, fixed in acetone at –20°C for 30 min, then air-dried again. GGT activity was detected histochemically by a modification of a previously described method[3] as follows. Sections were incubated for 1 h in a freshly prepared solution containing gamma-glutamyl-4-methoxy-2-naphthylamide (Sigma Chemical Co, St Louis, MO, U.S.A.) as substrate, glycylglycine as acceptor and Fast Blue BB salt as the diazonium coupling agent. After incubation, sections were rinsed in 0·85% saline, and then in 0·1 M cupric sulphate to allow the azo dye to chelate with cupric ions to form an insoluble red dye. Sections were counterstained with methyl green and mounted in glycerol. Material incubated in acceptor-free solutions and heat-inactivated sections was used as negative controls. Normal human kidney sections were used as positive controls.

Results

Adult normal skin

Adult normal interfollicular epidermis showed no GGT activity in serial sections of all specimens. GGT activity could only be found in the inner root sheath of anagen hair follicles (Fig. 1) and in eccrine sweat glands as previously reported.[15] There were two populations of cells within the eccrine gland, one of which was positive for GGT, the other negative. These GGT-positive cells possibly correspond to the clear cells seen in sections stained with haematoxylin and eosin although it was impossible with frozen tissue to identify these with certainty. Sweat ducts were negative. Sebaceous glands were negative in all the specimens studied, even on serial sections. A constant characteristic of normal skin was the presence of scattered GGT-positive cells in the dermis. Some of these cells were dendritic whilst others had a rounded appearance and when correlated with serial sections stained with chloroacetate esterase or toluidine blue, these cells appeared to be mast cells. Mast cells in the dermis of three cases of urticaria pigmentosa which we examined also expressed strong GGT activity. Similar GGT positive cells which have been found by other authors in the muscularis mucosa of stomach and intestinal wall have also been shown to be mast cells.[19]

Foetal skin

In foetal skin, GGT activity was detectable in the epidermis from 7 weeks' gestational age onwards; the enzyme could not be detected histochemically in two foetuses of 5 weeks' gestation. At the 7th week (Fig. 2), the very fragile primitive epidermis was GGT positive, although the subepidermal corium was negative. By 11 weeks the intensity of enzyme activity had considerably increased (Fig. 3); the basal layer expressed the enzyme very strongly while there was faint activity in the periderm. At 14 weeks, the basal layer labelling had diminished somewhat, although enzyme expression in the periderm persisted. After 14 weeks, the basal layer continued to progressively lose activity. In the later stages, at the 19th–20th week, we observed marked diminution of GGT activity of the basal layer; only the periderm, which is gradually shed, exhibited enzymatic activity (Fig. 4). Expression of the enzyme was totally lost by 24 weeks.

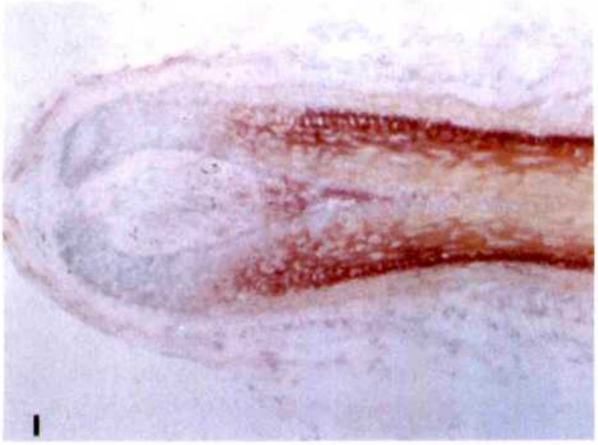

Figure 1. Anagen hair follicle. There is intense enzyme activity in the inner root sheath.

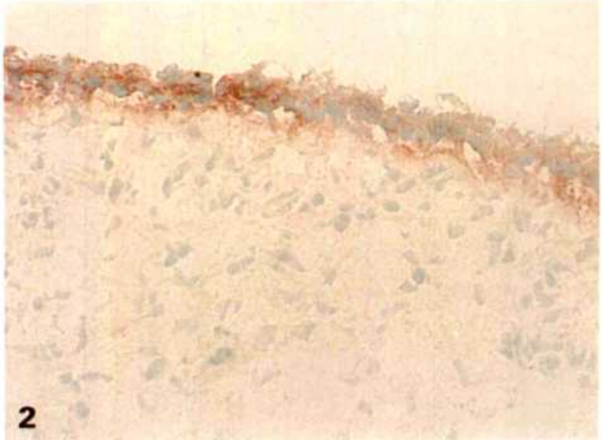

Figure 2. Foetal skin, 7 weeks. The fragile epidermis shows weak GGT expression, but the subepidermal corium is negative.

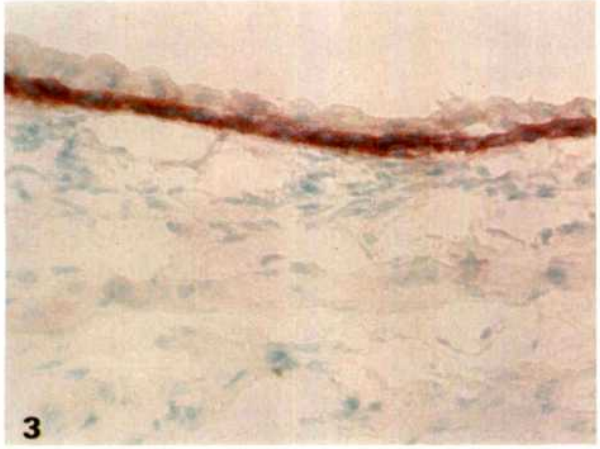

Figure 3. Foetal skin, 11 weeks. There is now intense GGT expression by the basal layer and the periderm shows weak positivity.

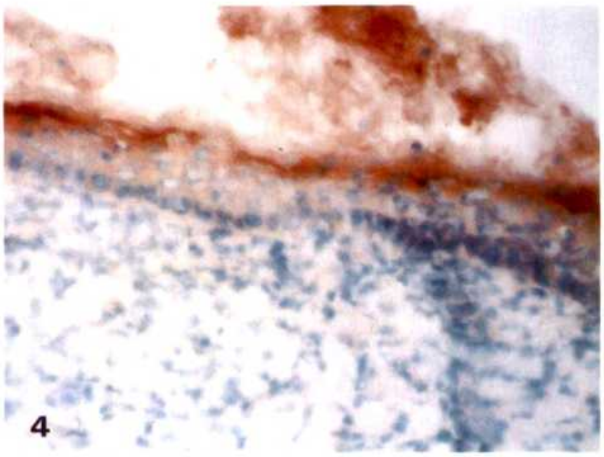

Figure 4. Foetal skin, 19 weeks. Basal cells are GGT negative. There is residual activity in the periderm.

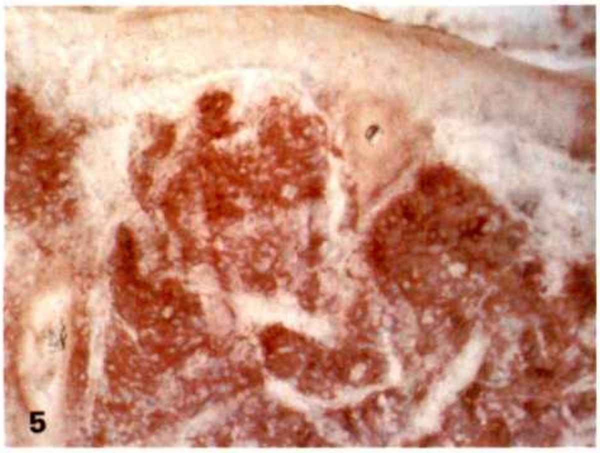

Figure 5. Squamous cell carcinoma. These tumours invariably strongly expressed GGT activity. Note that the overlying epidermis is negative.

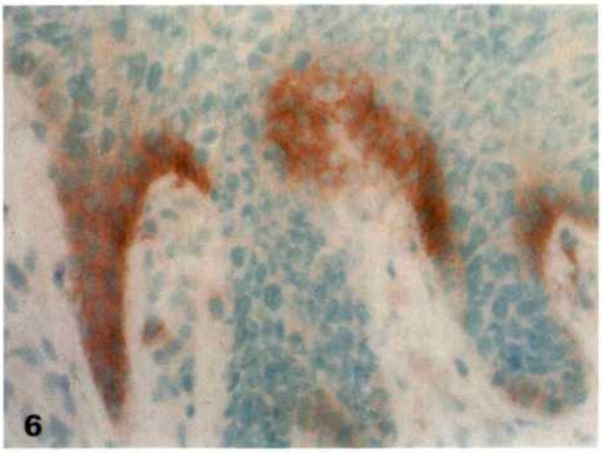

Figure 6. Bowen's disease. Focal clumps of keratinocytes express GGT although much of the dysplastic epidermis is GGT negative.

Benign proliferative lesions and inflammatory dermatoses

In all the inflammatory dermatoses examined there was a complete absence of GGT activity in the interfollicular epidermis. This included conditions known to exhibit keratinocyte hyperproliferation, such as psoriasis. With the exception of keratoacanthoma, all benign neoplasia failed to express GGT. All keratoacanthomas were positive for GGT although reactivity seemed less intense than in squamous cell carcinomas.

Malignant and premalignant lesions

All cases of squamous cell carcinoma were positive, showing GGT activity on the membrane of dysplastic cells both *in situ* and in invasive clusters of malignant cells, where the staining was most intense at the periphery of masses of squamous epithelium (Fig. 5). The intensity of the reaction product varied between tumours though we could not establish a relationship between this and the degree of dysplasia of single cells or tumour differentiation. Basal cell carcinomas were devoid of enzyme activity. Bowen's disease showed variable patterns of positive enzyme activity, which was either scattered throughout the epidermis or localized in focal areas (Fig. 6). Actinic keratoses showed on serial sections focal areas of activity, corresponding to the abnormal keratinocytes found on serial haematoxylin and eosin sections.

In malignant melanoma, GGT was absent in both epidermal malignant melanocytes and dermal tumour masses. However, epidermal keratinocytes in the immediate vicinity of melanoma cells were positive. The GGT positivity of epidermis was diffuse and limited laterally to the extent of the malignant infiltration.

As expected from the known GGT positivity of mammary carcinoma, intraepidermal mammary Paget's cells revealed the presence of the enzyme; however, the surrounding keratinocytes were negative.

Discussion

We have demonstrated that GGT is histochemically detectable in foetal epidermis from the 7th to 20th weeks of gestation. It is possible that the enzyme is present in younger foetuses, but at levels too low for histochemical detection. As epidermal development proceeds GGT is progressively limited to the more superficial epidermal layers. The disappearance of GGT activity by the 20th week coincides with major changes in the differentiation state of developing skin, such as the loss of keratin 18,[20] and tends to support the concept that GGT reflects epithelial differentiation. GGT remains undetectable in normal interfollicular epidermis in adult life, with the exception of skin appendages. Its physiological role is uncertain, although some authors suggest that GGT could be involved in the transport of molecules to or from active follicles.[21]

It seems clear that the factors governing GGT expression are complex. It cannot be regarded as a simple marker of cutaneous malignancy as it is present in normal mast cells, hair follicles and sweat glands. Neither can it depend solely on proliferation or metabolic rate, as the normal basal layer and psoriasis were uniformly negative. The most probable hypothesis is that GGT expression is a marker of cell differentiation, lost in embryogenesis by some cell lineages but retained by others. That malignancy is a state of de-differentiation is widely accepted and it seems that malignant keratinocytes behave like many other cells in their 'foetal' expression of GGT. The changes seen by us in epidermal tumours agree with previous reports[15] with the exception that all keratoacanthomas in our study were GGT positive; this may be due to the longer incubation time of 1 h used in our study. Certain of our findings therefore conflict with current concepts of what constitutes epidermal malignant disease. Absence of the enzyme in basal cell carcinomas and its presence in keratoacanthomas can only be reconciled if the former are considered to be hamartomatous[22] and the latter to be self-healing malignancies.[23] Although GGT does not appear in malignant melanocytes an unexpected finding is the expression of GGT by keratinocytes in close proximity to melanoma cells. This suggests that enzyme expression in keratinocytes may be induced by a soluble melanoma-associated factor.

Before GGT can be regarded as a useful histological marker for distinguishing malignant lesions from benign, more extensive assessment will be required. In particular, the GGT profiles of adnexal tumours and the induction of keratinocyte GGT by melanomas requires further study. Nonetheless, this enzyme may well be of importance in elucidating pathogenetic mechanisms underlying epidermal cell transformation in cutaneous carcinogenesis.

Acknowledgment

We are most grateful to Glaxo Laboratories for their generous assistance with the cost of colour reproduction.

References

1 Huseby NE. Subcellular localisation of gammaglutamyl transferase activity in guinea pig liver. Effect of phenobarbital on the enzyme activity levels. *Clin Chim Acta* 1979; **94**: 163–71.

2 Ratanasavanh D, Tazi A, Galteau MM, Siest G. Localization of gammaglutamyl transferase in subcellular fractions of rat and rabbit liver: effect of phenobarbital. *Biochem Pharmacol* 1979; **28**: 1363–5.

3 Rutenburg AM, Kim H, Fishbein JW *et al.* Histochemical and ultrastructural demonstration of GGT activity. *J Histochem Cytochem* 1969; **17**: 517–26.

4 Albert AZ, Orlowski M, Szewczuk A. Histochemical demonstration of gammaglutamyl transpeptidase. *Nature* 1961; **191**: 767–8.

5 Orlowski M, Meister A. The gamma glutamyl cycle: A possible transport system for amino-acids. *Proc Natl Acad Sci USA* 1970, **67**: 1248–55.

6 Curthoys NP, Hughey RP. Characterisation and physiological function of rat renal gamma glutamyl transpeptidase. *Enzyme* 1979; **24**: 383–403.

7 Fiala S, Trout E, Pragani B *et al.* Increased gammaglutamyl transferase activity in human colon cancer. *Lancet* 1979; **i**: 1145.

8 Groscurth P, Fleming N, Kistler GS. The activity and distribution of gammaglutamyl transpeptidase in human lung cancers serially transplanted in nude mice. *Histochemistry* 1977; **53**: 135–42.

9 Kalengayi MMR, Ronchi G, Desmet VJ Histochemistry of gammaglutamyl transpeptidase in rat liver during aflatoxin B1-induced carcinogenesis. *J Natl Cancer Inst* 1975; **55**: 579–88.

10 Aronsen KF, Nosslin B, Pihl B. The value of gammaglutamyl transpeptidase as a screen for liver tumours. *Acta Chir Scand* 1970; **136**: 17–22.

11 Szczeklic E, Orlowsky M, Szewczuk A. Serum gammaglutamyl transpeptidase activity in liver disease. *Gastroenterology* 1961; **41**: 353–9.

12 DeYoung LM, Richards WL, Bonzelet W *et al.* Localisation and significance of gammaglutamyl transpeptidase in normal and neoplastic mouse skin. *Cancer Res* 1978; **38**: 3697–701.

13 Aldaz CM, Conti CJ, Larcher F *et al.* Sequential development of aneuploidy, keratin modifications and gammaglutamyl transferase expression in mouse skin papillomas. *Cancer Res* 1988; **48**: 3253–7.

14 Chiba M, Maley MA, Klein-Szanto AJP. Sequential study of gammaglutamyl transferase during complete and two stage mouse skin carcinogenesis. *Cancer Res* 1986; **46**: 259–63.

15 Chiba M, Jimbow K. Expression of gamma glutamyl transpeptidase in normal and neoplastic epithelial cells of human skin: a marker for differentiation of malignant epithelial tumours. *Br J Dermatol* 1986; **114**: 459–64.

16 Sirica AE, Richards W, Tsukada Y *et al.* Fetal phenotypic expression by adult rat hepatocytes on collagen gel/nylon meshes. *Proc Natl Acad Sci USA* 1979; **76**: 283–7.

17 Buxman MM, Kocarnik MJ, Holbrook KA. Differentiation markers in fetal epidermis: transglutaminase and transpeptidase. *J Invest Dermatol* 1979; **72**: 171–7.

18 Streeter GL. Foot length of the foetus. *Contributions to Embryology* 1921; **55**: 143.

19 Albert Z, Rzucidlo Z, Starzyk H. Biochemical and histo-chemical investigations of the gammaglutamyl transpeptidase in embryonal and adult organs of man. *Acta Histochem* 1970; **37**: 74–9.

20 Lane EB, Bartek J, Purkis PE, Leigh IM. Keratin antigens in differentiating skin. *Ann NY Acad Sci* 1985; **455**: 241–58.

21 Richards WL, Astrup EG. Expression of GGT activity in the developing mouse tooth, intervertebral disc and hair follicle. *Cancer Res* 1982; **42**: 4143–48.

22 Lever WF, Schaumburg-Lever G. Basal cell epithelioma. In: *Histopathology of the Skin* (Lever WF, Schaumburg-Lever G, eds), 6th edn. J. B. Lippincott & Co, 1983; 562.

23 Rook A, Whimster IW. Keratoacanthoma: a 30 years retrospective. *Br J Dermatol* 1979; **100**: 41–7.

British Journal of Dermatology (1991) 125, 6–8. ADONIS 000709639100154U

The induction of epidermal ornithine decarboxylase following tape stripping is inhibited by a topical vitamin D_3 analogue (MC903)

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Accepted for publication 21 January 1991

Summary

The efficacy of MC903, a vitamin D_3 analogue, in reducing hyperproliferation as determined by levels of ornithine decarboxylase (ODC) was investigated in a double-blind study of 15 patients with chronic plaque psoriasis. The lesions of psoriasis were treated for 8 weeks with MC903 in one of two different cream bases or with a placebo cream. Biopsies were taken before and after treatment. In addition an uninvolved area of skin was treated during the last 3 weeks and this as well as control areas were then sellotape stripped and biopsied after 8 h. Clinical improvement was seen in eight out of 11 patients treated with MC903 but there was no reduction in the level of ODC in psoriatic lesions after 8 weeks of treatment. The levels of ODC in the tape-stripped uninvolved skin after 3 weeks of treatment with MC903 averaged $22{\cdot}5 \pm 4{\cdot}2$ pmol/min/mg protein as compared to $58{\cdot}6 \pm 12{\cdot}6$ pmol/min/mg protein ($P = 0{\cdot}004$). The trauma-induced induction of ODC activity was markedly inhibited by the application of MC903.

The skin is the site for the synthesis of vitamin D and the target tissue for its active metabolite 1,25 dihydroxyvitamin D_3 ($1{,}25(OH)_2D_3$). This hormone inhibits proliferation and induces terminal differentiation of keratinocytes both *in vitro* and *in vivo*.[1] Topical and oral administration of $1{,}25(OH)_2D_3$ has been used in the treatment of psoriasis.[1] The administration of this calcium-regulating hormone may result in hypercalcaemia and hypercalcuria. MC903 is a vitamin D_3 analogue that is equally potent in the epidermis, but 100 times less active in its calciotropic effect.[2] This analogue used in an ointment base has proved to be effective and safe in the treatment of psoriasis.[3–5]

Ornithine decarboxylase (ODC) is a rate-limiting enzyme for polyamine biosynthesis. Because polyamines are required before DNA synthesis can begin, ODC is an appropriate 'marker enzyme' for cellular proliferation.[6] In normal epidermis the level of ODC is undetectable, but ODC induction occurs following injury such as tape stripping[7] and UV-irradiation.[8] Also the application of tumour-promoting agents[9] can induce ODC activity. Tape stripping of human epidermis causes a peak in the level of ODC at 8 h.[10] Several groups have reported increased ODC levels in lesions of psoriasis and have shown that these become normal following treatment.[11] It has been found that $1{,}25(OH)_2D_3$ can inhibit the induction of ODC in the epidermis of rodents,[12,13] but to our knowledge it has not been reported in humans. The aim of the present study was to investigate the effect of MC903 on the level of ODC in lesions of psoriasis and to determine its influence on the induction of this enzyme with tape stripping.

Methods

Subjects and treatment protocol

Fifteen patients with chronic plaque psoriasis (eight male, seven female) with a mean age of 39 years participated in this double-blind study to evaluate the efficacy of two different MC903 creams. In cream A, MC903 was dissolved with an emulgator and in cream B, MC903 was suspended as fine particles. Both creams contained MC903 in a concentration of 50 μg/g.

In the 2 weeks prior to treatment the patients used only a cream base. Six were randomly allocated to cream A and six to cream B, and three used the placebo cream. The creams were applied to all the lesions twice daily over a period of 8 weeks, apart from those on the scalp, face and anogenital region. No other treatment was permitted.

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Skin sampling and tape stripping

Biopsies approximately 1 cm^2 were taken from the centres of active lesions on the right elbow using the Castroviejo keratotome set at a depth of 0·3 mm on the first and last days of treatment. The patients were also instructed to apply the cream to an uninvolved area of skin on the left upper arm during the last 3 weeks of treatment, and this area and an untreated area on the right arm were then stripped with Sellotape®, as previously described.[14] After 8 h, keratotome biopsies were taken from the two sites. The biopsies were snap frozen in liquid nitrogen and stored at −80°C.

ODC and protein measurement

The measurement of ornithine decarboxylase and soluble protein levels were carried out as previously described.[10] Shave biopsies were homogenized in buffer and aliquots incubated at 37°C after adding L-(1-^{14}C)-ornithine. Each tube was fitted with a plastic cap carrying a filter paper moistened with potassium hydroxide; the reaction was then stopped with hydrochloric acid, incubated again and the $^{14}CO_2$ measured. The soluble protein was determined in the clear supernatant by direct fluorescence.

Statistical analysis

Analysis of variance was carried out for treated and untreated skin using the SAS package (*t*-test for paired data) and a VAX computer system.

Results

Treatment with MC903 cream over a period of 8 weeks produced virtual clearing of the psoriasis in three patients and five showed a moderate reduction in the extent of the lesions. In three patients the lesions appeared unchanged. One patient was not included due to the use of a solarium during the trial. In the placebo group, all three patients had a worsening of their psoriasis.

In the ornithine decarboxylase assay the reagent blank, including the background of the instrument, averaged about 22 c.p.m.; the total reaction samples were between 60 and 950 c.p.m. The assay was linear with respect to time over 45 min, and an incubation period of 45 min was used for the routine measurements. The reproducibility was good with duplicate assays averaging within ±4·1% of the mean.

The levels of ODC in the psoriatic lesions ($n = 12$) before treatment averaged 2·6 ± 0·7 pmol/min/mg protein (mean ± SEM). This agrees with a previous report[15] in which ODC levels of 2·7 pmol/min/mg protein were found. Levels of ODC after treatment with MC903 ($n = 11$) in creams A and B were 3·1 ± 1·3 and 2·4 ± 0·6 pmol/min/mg protein, respectively. Levels of ODC after treatment with placebo ($n = 2$) were 3·2 and 6·2 pmol/min/mg protein. The present study did not show any down-regulation of ODC to basal and undetectable levels.

The level of ODC in uninvolved and untreated skin after tape stripping was 58·6 ± 12·6 pmol/min/mg protein ($n = 11$). Treatment with MC903 ($n = 9$) resulted in a substantial reduction in the level of ODC in the cream A group to 26·6 ± 6·8 ($P = 0·06$) and in cream B group to 17·3 ± 3·4 ($P = 0·07$). Pooling of all the treated samples gave a value of 22·5 ± 4·2 pmol/min/mg protein, a highly significant difference from the controls ($P = 0·004$). The values of ODC after treatment with placebo in two patients were 137·2 and 80·4 pmol/min/mg protein, excluding any effect by the base cream.

Discussion

Although treatment with MC903 resulted in a clinical improvement in eight of the 11 patients, the pretreatment and post-treatment levels of ornithine decarboxylase were similar. However, treatment with MC903 of uninvolved skin resulted in a marked inhibition of the induction of ODC following standardized injury.

MC903 has been shown to inhibit synthesis of DNA[2,16] and to reduce the amount of keratin 16, a marker for hyperproliferation of keratinocytes,[16,17] and to reduce the number of cycling cells as indicated by nuclear staining of Ki67.[18] The lack of correlation between clinical resolution and the levels of ODC in the lesions of psoriasis after treatment with MC903 is surprising. This suggests that other mechanisms are involved in the resolution of the hyperproliferation seen in psoriasis. Following treatment with MC903 in psoriasis there is a disappearance of polymorphonuclear leucocytes.[18] The level of ODC could be a marker for the 'complete' healing of lesions in psoriasis.

Trauma-induced hyperproliferation is an acute event and maximal ODC induction is seen 8 h following tape stripping.[10] Forty-eight hours later the percentage of cells in the SG2M-phase reaches maximum levels.[14] Thus this is an appropriate model for studying the induction of hyperproliferation in the epidermis, and we found that this was markedly inhibited by the topical application of MC903. This supports the hypothesis that the steroid receptor superfamily is of relevance in the control of epidermal proliferation. Members of this family include the glucocorticoid, thyroid hormone, retinoic acid and vitamin D receptors. In their inactive state they are thought to be bound to an inhibitor protein that blocks the DNA-binding domain of the receptor. The binding of the hormone to the receptor causes the inhibitor protein to dissociate, with activation of the receptor. The receptor in turn is then capable of blocking or stimulating an enhancer/promoter region before or after a coding DNA sequence, for example ODC-mRNA.[19] There is recent evidence that the phosphorylated c-jun/c-fos product (AP-1) which is responsible for the activation of the ODC-promoter complexes with and is blocked by the activated corticosteroid receptor.[20] Thus glucocorticoids can block the induction of ODC in the epidermis.[21] Thyroid hormone stimulates the proliferation of epidermal cells,[22] probably by induction of ODC.[23] Retinoic acid inhibits the induction of ODC in the skin[7] and vitamin D_3 also blocks ODC induction in the epidermis.[12,13]

Acknowledgments

The authors thank Dr Paul Mier for helpful discussions, Dr Elke de Jong for assistance at the outset of the trial and Ms Zamira Arts for typing the manuscript.

References

1 Holick MF, Smith E, Pincus S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxy vitamin D_3. *Arch Dermatol* 1987; **123**: 1677–83.

2 Binderup L, Bramm E. Effects of a novel vitamin D analogue MC903 on cell proliferation and differentiation *in vitro* and on calcium metabolism *in vivo*. *Biochem Pharmacol* 1988; **37**: 889–95.

3 Kragballe K, Beck HI, Søgaard H. Improvement of psoriasis by a topical vitamin D3 analogue (MC903) in a double blind study. *Br J Dermatol* 1988; **119**: 223–30.

4 Staberg B, Roed-Petersen J, Menné T. Efficacy of topical treatment in psoriasis with MC903, a new vitamin D analogue. *Acta Derm Venerol (Stockh)* 1989; **69**: 147–50.

5 Kragballe K. Treatment of psoriasis by the topical application of the novel cholecalciferol analogue calcipotriol (MC903). *Arch Dermatol* 1989; **125**: 1647–52.

6 Lowe NJ. Epidermal ornithine decarboxylase, polyamines, cell proliferation and tumor promotion. *Arch Dermatol* 1980; **116**: 822–5.

7 Bouclier M, Shroot B, Dionisius V *et al.* A rapid and simple test system for the evaluation of the inhibitory activity of topical retinoids on sellotape stripping induced ODC activity. *Dermatologica* 1984; **169**: 242.

8 Lowe NJ, Verma AK, Boutwell RK. Ultraviolet light induces epidermal ornithine decarboxylase activity. *J Invest Dermatol* 1978; 71: 417–18.

9 Lichti U, Patterson E, Yuspa SM. Retinoic acid differentially inhibits the induction of ODC by TPA and by germicidal ultraviolet light. *Proc Am Assoc Cancer Res* 1979; **20**: 105.

10 Arnold WP, van Hooijdonk CAEM, Mier PD, van de Kerkhof PCM. Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin. *Br J Dermatol* 1990; **123**: 581–5.

11 Lowe NJ. Cutaneous polyamines and their biosynthetic enzymes. In: *Psoriasis* (Roenigk HH, Maibach HI, eds), New York: Dekker, 1985; 249–54.

12 Chida K, Hashiba H, Suda T, Kuroki T. Inhibition by 1a, 25-dihydroxy vitamin D_3 of induction of epidermal ornithine decarboxylase caused by TPA and teleocidin B. *Cancer Res* 1984; **44**: 1387–91.

13 Chida K, Hashiba H, Fuhushima M *et al.* Inhibition of tumor promotion in mouse skin by 1,25-dihydroxy vitamin D_3. *Cancer Res* 1985; **45**: 5426–30.

14 Van de Kerkhof PCM, van Rennes H, de Grood RM *et al.* Response of the clinically uninvolved skin of psoriatic patients to standardized injury. *Br J Dermatol* 1983; **109**: 287–94.

15 Russell DH, Combest WL, Duell EA *et al.* Glucocorticoid inhibits elevated polyamine biosynthesis in psoriasis. *J Invest Dermatol* 1978; **71**: 177–81.

16 De Mare S, de Jong EGJM, van de Kerkhof PCM. DNA content and Ks 8.12 binding of the psoriatic lesion during treatment with the vitamin D3 analogue MC903 and betamethasone. *Br J Dermatol* 1990; **123**: 291–5.

17 Holland DB, Roberts SG, Russell *et al.* Changes in epidermal keratin levels during treatment of psoriasis with the topical vitamin D3 analogue MC903. *Br J Dermatol* 1990; **122**: 284.

18 De Jong EMGJ, van de Kerkhof PCM. Simultaneous assessment of inflammation and epidermal proliferation in psoriatic plaques during longterm treatment with the vitamin D3 analogue MC903: modulations and interrelations. *Br J Dermatol* 1991; **124**: 221–9.

19 Alberts B, Bray D, Lewis J *et al.* Signaling mediated by intracellular receptors: mechanism of steroid hormone action. In: *Molecular Biology of the Cell.* New York & London: Garland Publ., 1989; 690–3.

20 Schüle R, Rangarajan P, Kliewer S *et al.* Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. *Cell* 1990; **62**: 1217–26.

21 Lowe NJ, Breedings J. Anti-inflammatory drug effects on ultraviolet light induced epidermal ornithine decarboxylase and DNA synthesis. *J Invest Dermatol* 1980; **74**: 418–20.

22 Holt PJA. *In vitro* responses of the epidermis to triiodothyronine. *J Invest Dermatol* 1978; **71**: 202–4.

23 Ruel J, Chenard C, Coulombe P, Dussault JH. Thyroid hormones modulate ornithine decarboxylase in the immature rat cerebellum. *Can J Physiol Pharmacol* 1984; **62**: 1279–83.

British Journal of Dermatology (1991) 125, 9–13. ADONIS 0007096391001558

In-vitro DNA synthesis of keratinocytes in normal human skin, psoriasis, seborrhoeic keratosis, Bowen's disease and basal cell carcinoma

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Accepted for publication 3 January 1991

Summary

The DNA-synthesizing cells in benign and malignant keratotic diseases including psoriasis, seborrhoeic keratosis, Bowen's disease and basal cell carcinoma were studied. Cells in S-phase were labelled after small pieces of the lesions were incubated in tissue culture medium that contained 5-bromo-2′-deoxyuridine (BrdU). The labelling of DNA was analysed using the labelling index and pattern. Although the malignant diseases tended to show a higher DNA labelling index, neither this nor the loss of polarity alone was associated with malignancy. However, a higher DNA labelling index together with a loss of polarity was characteristic for malignancy.

The cell kinetics of human tumours have been studied by the administration of tritiated thymidine prior to biopsy or excision.[1] This requires the administration of a radioactive substance to the patient and the subsequent visualization of the incorporated tritiated thymidine by autoradiography. There are only a limited number of cell kinetic studies of human epidermis and epidermal tumours and because of the different methods used in the labelling and counting of the cells, it is difficult to compare directly the labelling indices for the various conditions.[2–6] A recent report[7] showed that excised tumours synthesized DNA at a rate comparable to that *in vivo* when they were incubated at a high pressure in 95% O_2 and 5% CO_2. The development of a monoclonal antibody against 5-bromo-2′-deoxyuridine (BrdU) has enabled the detection of this thymidine analogue using an immunohistochemical method.[8] We used BrdU labelling *in vitro* under hyperbaric conditions to study DNA labelling of psoriasis, seborrhoeic keratosis, Bowen's disease, basal cell carcinoma (BCC) and normal skin.[9]

Methods

Tissue

The cutaneous tumours were excised under local anaesthesia and cut into small pieces less than 1 mm^3 in phosphate-buffered saline (PBS) at 4°C.

BrdU labelling

Small specimens were placed in a glass vial containing nucleoside- and nucleotide-free α-MEM with 10% heat-inactivated fetal calf serum with 100 μM BrdU. The vial was tightly sealed with a Teflon rubber cap after replacing the air with 95% O_2 and 5% CO_2 at three atmospheres of pressure and incubated in a shaking water bath for 1 h at 37°C.[7] Preliminary studies carried out on mice showed that the DNA labelling index of normal skin using this *in-vitro* method was about the same as that from pulse labelling *in vivo* with 100 mg/kg of BrdU given intraperitoneally. The DNA labelling index in normal human skin and in seborrhoeic keratosis was lower and the staining of each nucleus weaker when the incubation took place at one atmospheric pressure, indicating the necessity for hyperbaric conditions.

Immunohistochemistry

The labelled tissues were fixed in 70% ethanol overnight at 4°C and then embedded in paraffin. Sections 5 μm thick were cut and the DNA denatured by incubation in 2 N HCl for 1 h at 37°C and then were washed in PBS at pH 9 and in PBS at pH 7·4.[10] The incorporated BrdU was visualized using an anti-BrdU monoclonal antibody. The sections were incubated with this antibody in a 1:100 dilution for 60 min in a moist chamber at room temperature. After washing with PBS the sections were then incubated with biotinylated goat anti-mouse IgG antibody for 20 min and followed by a wash with PBS. The sections were then incubated with peroxidase-

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conjugated streptavidin for 20 min and then washed thoroughly with PBS. The antibody-binding sites were then visualized with diaminobenzidine. The labelled cells were counted by two observers and the DNA labelling index expressed as labelled epidermal cells/epidermal cells (basal and spinous layers) × 100.[3] Each value was expressed as mean ± standard error.

Materials

BrdU was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.) and the anti-BrdU monoclonal antibody from Becton Dickinson (Mountain View, CA, U.S.A.). The nucleoside- and nucleotide-free α-MEM came from Gibco (Grand Island, NY, U.S.A.). A streptavidin immunohistochemical staining kit was obtained from BioGenex Laboratories (Dublin, CA, U.S.A.).

Results

The cells in the basal layer and the immediate suprabasal layer of the epidermis in the normal skin were labelled with BrdU (Fig. 1). The DNA labelling index of the epidermis was 1·4 ± 0·4%. The basal layer in the sebaceous gland on facial skin had a higher DNA labelling index than that of interfollicular epidermis in adults (data not shown).

Plaque lesions of psoriasis obtained from four patients were studied and the labelled cells were mostly in the basal and suprabasal layers of the epidermis, although a few labelled cells were present in the lower spinous layer (Fig. 2). The DNA labelling index was 9·3 ± 1·4%.

Seborrhoeic keratoses excised from five patients were studied and the labelled cells were mostly seen in the basal and suprabasal layers; however, in some tumours the labelled cells were nearer to the surface (as shown on Fig. 3). The DNA labelling index was 1·2 ± 0·3%.

In Bowen's disease, the labelled cells were observed not only in the basal and lower spinous layers but were also found much higher up (Fig. 4). These labelled cells were sometimes found aggregated together (Fig. 4 inset). The DNA labelling index was 7·7 ± 1·4%.

The nuclei in the BCCs excised from five patients showed a random labelling with BrdU. Not only were the cells at the margins labelled but also those inside the islands of tumour (Fig. 5). The DNA labelling index was 8·5 ± 1·2%.

The results of the DNA labelling index and pattern are summarized in Table 1.

Discussion

To overcome the drawbacks of tritiated thymidine studies of DNA synthesis in skin disorders and tumours, we have used an *in-vitro* method of incubation with 100 μM BrdU under three atmospheres of pressure in 95% O_2 with 5% CO_2 as previously reported.[7] Cell cycle progression was not blocked and comparable cell cycle kinetics were obtained using this method.

We found that a high DNA labelling index alone did not correlate directly with malignancy because psoriasis also had a high index. The relationship between loss of polarity in labelling and malignancy was also studied, and the polarity in labelling in BCC was lost and labelled cells were to be found well within the tumour islands. In psoriasis the labelled cells were mostly restricted to the basal and suprabasal layers despite the high DNA labelling index. Although some labelled cells in seborrhoeic keratosis were to be found well away from the basal layer most of the labelled cells were in or near the basal layer. Thus we conclude that while a high DNA

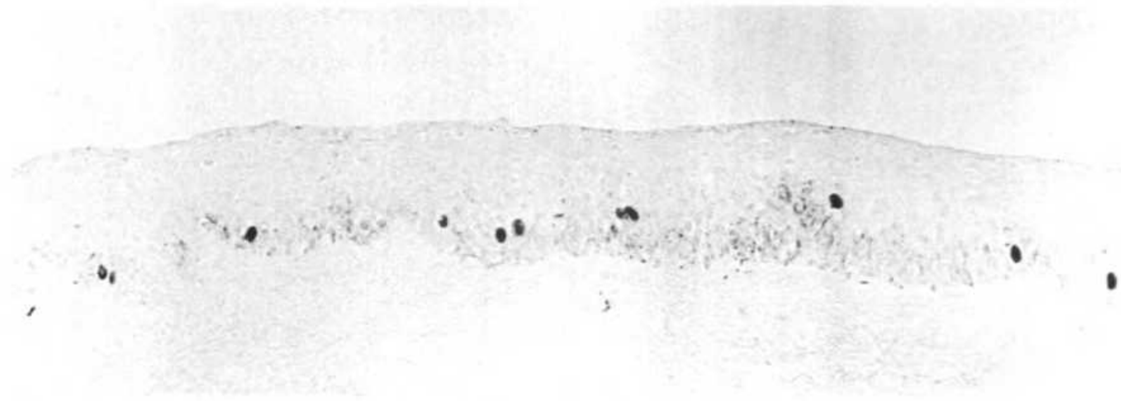

Figure 1. Normal skin showing labelled cells in the basal and suprabasal layers of the epidermis.

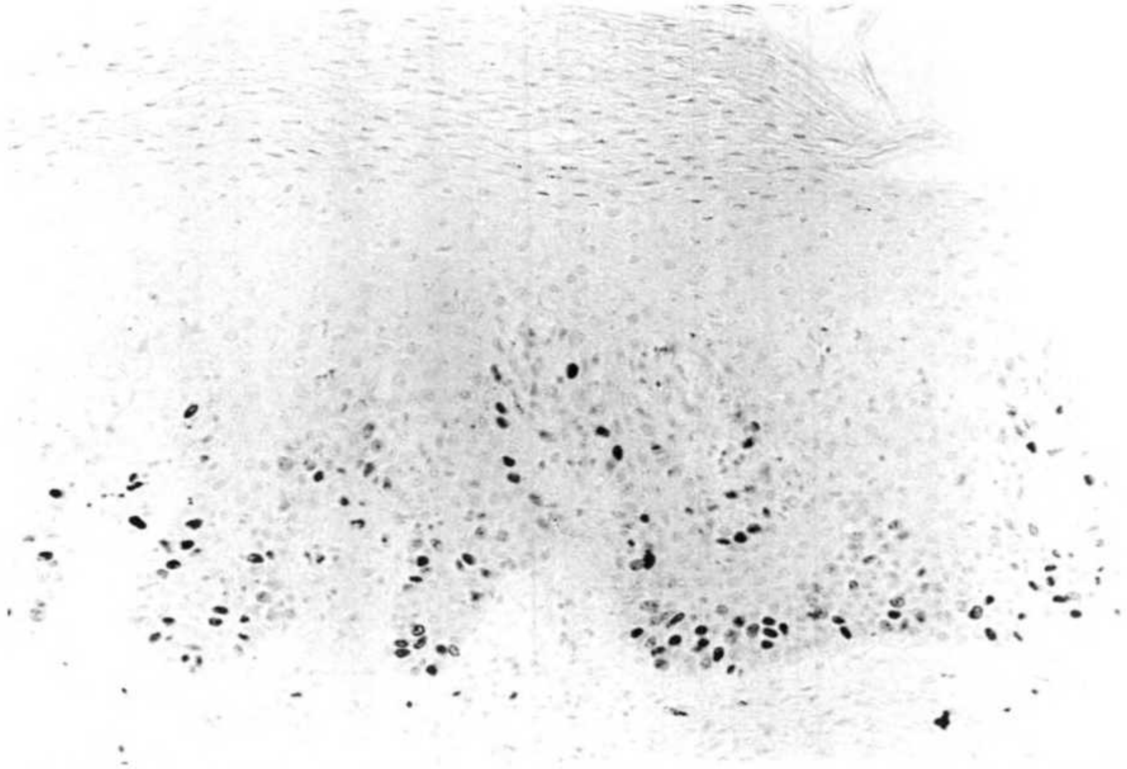

Figure 2. Psoriatic skin showing high labelling index of BrdU largely in the basal and suprabasal layers of the epidermis with a few labelled cells in the lower spinous layers.

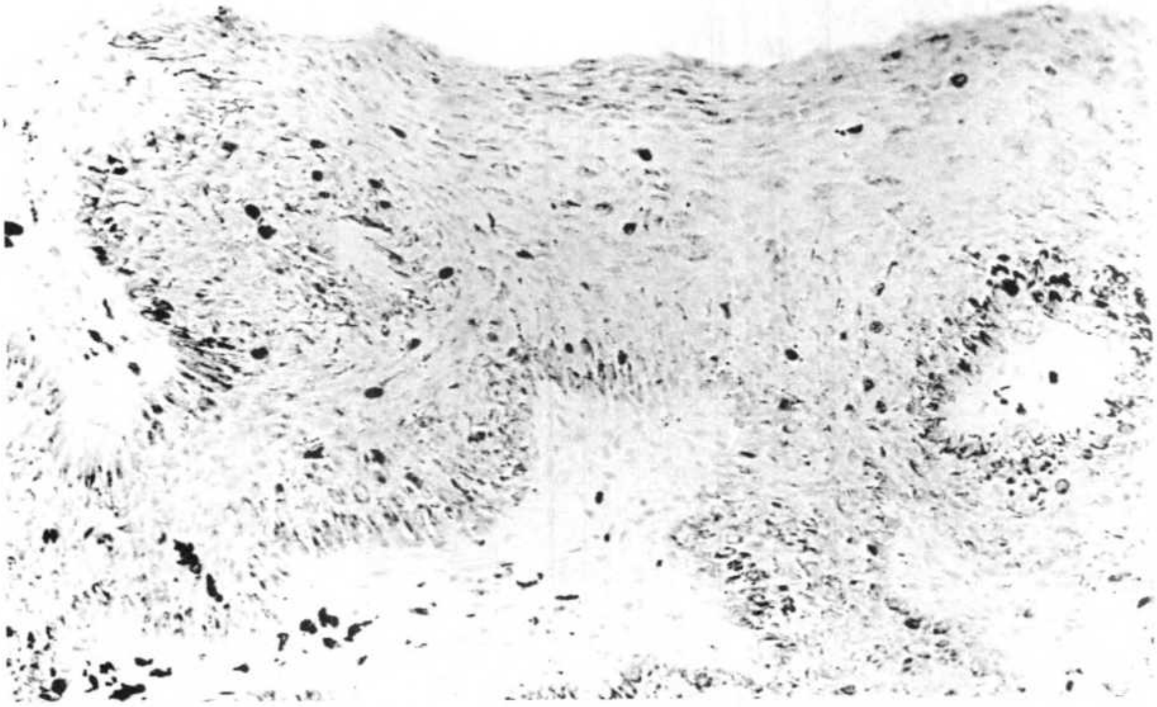

Figure 3. DNA labelling index was low in seborrhoeic keratosis, although labelled cells were not restricted to the basal or suprabasal layers of the epidermis.

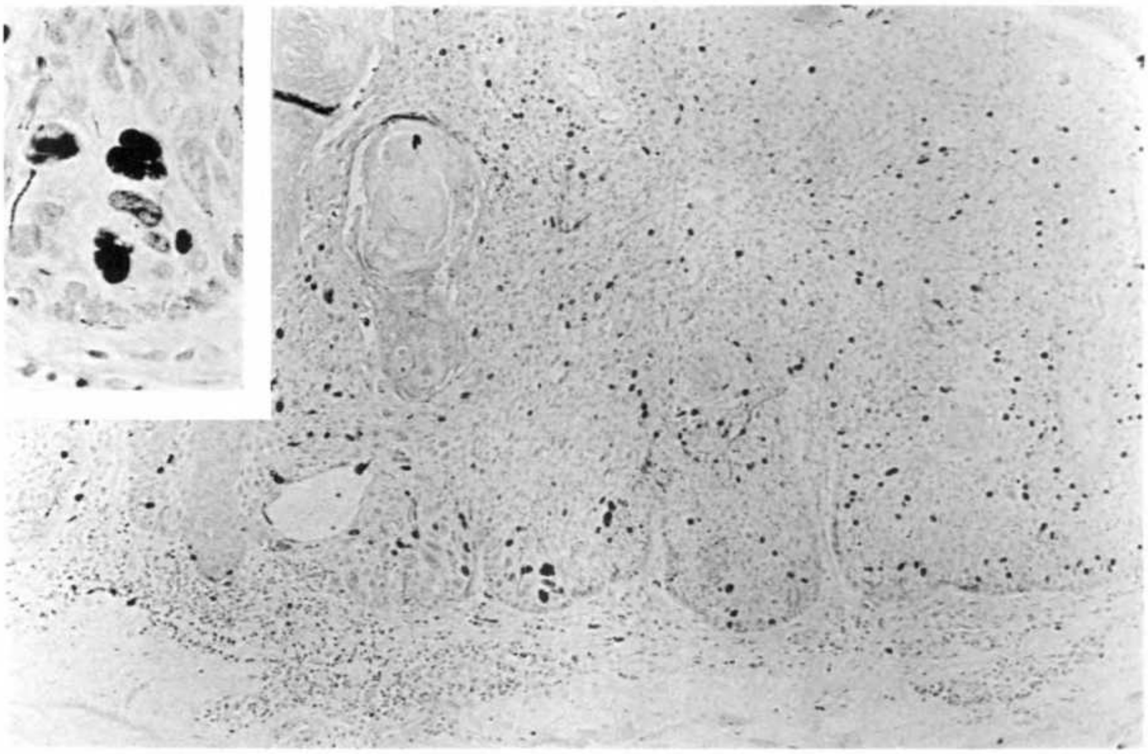

Figure 4. Labelled cells present throughout the epidermis in Bowen's disease, showing loss of polarity in labelling. Inset: aggregated cells labelled with BrdU.

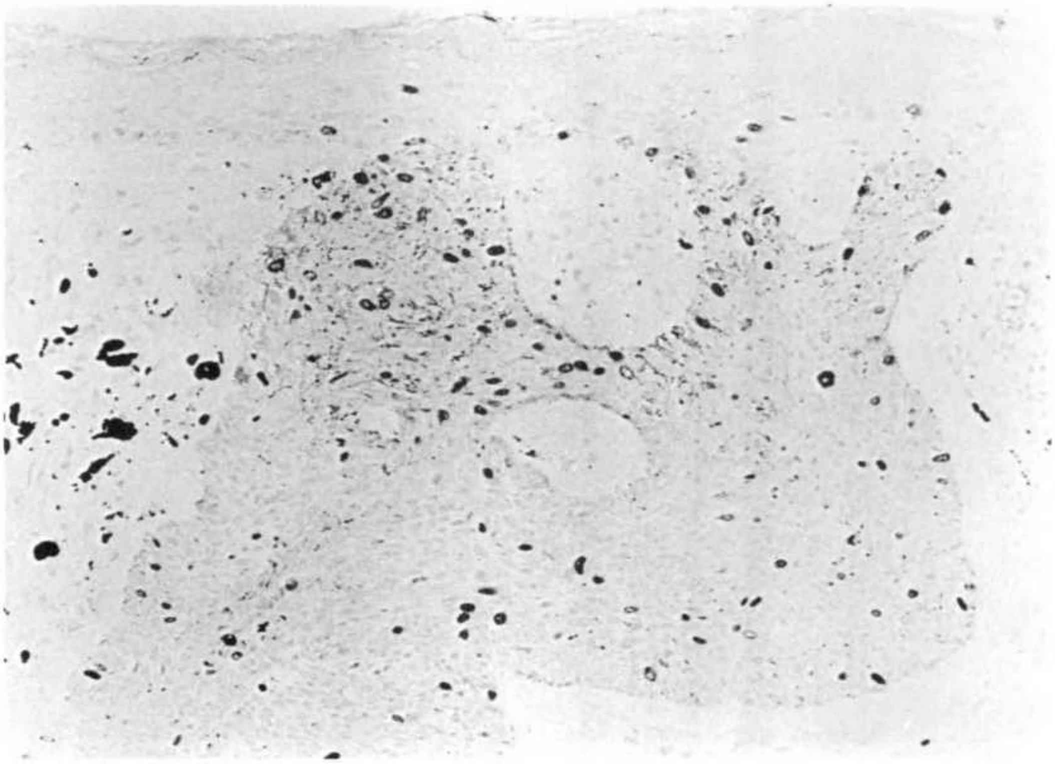

Figure 5. Labelled cells scattered throughout the tumour in BCC.

Table 1. Labelling index and labelling pattern in benign and malignant keratotic diseases

Disease (no. of cases)	Benign or malignant	Labelling index	Polarity in labelling
Normal skin (4)	Benign	1·4±0·4%	++
Psoriasis (4)	Benign	9·3±1·4%	++
Seborrhoeic keratosis (5)	Benign	1·2±0·3%	+
Bowen's disease (10)*	Malignant	7·7±1·4%	+
Basal cell carcinoma (5)	Malignant	8·5±1·2%	–

++, labelled cells were restricted to the germinative layer and its vicinity; +, many scattered labelled cells but germinative layer shows a predominance in DNA labelling; –, germinative compartment was not preferentially labelled.

*, 10 lesions from four patients were studied.

labelling index or loss of polarity in labelling alone are not specific for malignant disease, together they appeared to be more characteristic for malignancy.

Basal cell carcinoma is a locally invasive tumour that arises from the basal cells of the epidermis. It is known that BCC requires the dermal stroma for growth.[11] It has been suggested that metastases do not arise from this tumour because the cells require contact with the stroma for DNA synthesis and cell division. However, this is unlikely because our study shows DNA is synthesized by cells well inside the tumour islands and away from the surrounding stroma. This agrees with *in-vivo* labelling with tritiated thymidine when the labelled cells were to be found scattered within the tumour epithelial masses.[4] There have been no previous reports on cell kinetics in seborrhoeic keratosis. It is of interest that the BrdU-labelled cells were to be found near the surface and well away from the basal layer. The cells in seborrhoeic keratosis had a basaloid appearance like that of BCC and an ability to synthesize DNA without being in contact with the basement membrane or the surrounding dermal stroma.

Acknowledgment

This study was supported by a grant from the Ministry of Education and Culture of Japan (02670484) to K.S. We thank Mr Steven Wright for checking the English of the manuscript.

References

1 Weinstein GD. Epidermal cell kinetics. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K *et al.*, eds), 3rd edn, Vol 1. New York: 1987; 154–65.

2 Weinstein GD, Frost P, Abnormal cell proliferation in psoriasis. *J Invest Dermatol* 1968; 50: 254–9.

3 Lachapelle JM, Gillman T. Tritiated thymidine labelling of normal human epidermal cell nuclei. *Br J Dermatol* 1969; 81: 603–16.

4 Weinstein GD, Frost P. Cell proliferation in human basal cell carcinoma. *Cancer Res* 1970; 30: 724–80.

5 Goodwin P, Hamilton S, Fry L. The cell cycle in psoriasis. *Br J Dermatol* 1974; 90: 517–24.

6 Duffill M, Wright N, Shuster S. The cell proliferation kinetics of psoriasis examined by three *in-vivo* techniques. *Br J Dermatol* 1976; 94: 355–62.

7 Sasaki K, Takahashi M. Preservation of cell cycle characteristics in solid tumors *in vitro*. *Cancer Res* 1980; 40: 4810–2.

8 Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. *Science* 1982; 218: 474–6.

9 Sasaki K, Ogino T, Takahashi M. Immunological determination of labelling index on human tumor tissue sections using monoclonal anti-BrdU antibody. *Stain Technol* 1986; 61: 155–61.

10 Dolbeare F, Gratzner H, Pallavicini MG, Gray JW. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. *Proc Natl Acad Sci USA* 1983; 80: 5573–7.

11 Pollack SV, Goslen JB, Sherertz EF, Jegasothy BV. The biology of basal cell carcinoma: a review. *J Am Acad Dermatol* 1982; 7: 569–77.

British Journal of Dermatology (1991) 125, 14–17.　ADONIS 000709639100156M

Increased tryptase levels in suction-blister fluid from patients with urticaria

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Accepted for publication 28 January 1991

Summary

The levels of tryptase in the suction-blister fluid from patients with chronic urticaria, urticaria pigmentosa, cholinergic urticaria, urticarial dermographism, prurigo of unknown origin, eczema, psoriasis, atopic dermatitis, and from healthy controls were studied. The blister fluid from controls contained up to 15 μg/l of tryptase, whereas that from patients with active urticaria contained > 50 μg/l. This study demonstrates that patients with urticaria have mast cells that readily release tryptase in both the lesional and non-lesional areas of skin.

Urticaria is a common disorder affecting up to 20% of the population. Histamine can induce urticaria and its release is of major significance in this condition, as indicated by the effectiveness of antihistamine therapy. The level of skin histamine is increased in urticaria[1] and in the suction-blister fluid of patients with cold urticaria.[2] However, histamine is metabolized rapidly and it is difficult to use the amount found as a quantitative parameter for mast cell degranulation in skin.

Human mast cells release serine proteinases, proteoglycans, prostaglandin D_2, and leukotriene C_4 as well as histamine upon degranulation and are the only significant source of the serine proteinases, tryptase and chymase. Skin mast cells contain an average of 35 pg of tryptase and 4·5 pg of chymase per cell (TC cells). The lung and intestinal mast cells contain only 10 pg tryptase per cell (T cells)[3,4] and the basophils contain only minute amounts of the enzyme (0·04 pg/cell).

Recently, an immunoassay was described[5] based on an ELISA method with a goat anti-tryptase polyclonal antibody as the detecting antibody and a monoclonal antibody as the catching antibody. In addition, newly developed monoclonal antibodies to tryptase have led to the introduction of a commercially available radioimmunoassay (RIA).[6,7] Increased serum levels of tryptase have been found in systemic anaphylactoid reactions and in 11 out of 17 patients with systemic mastocytosis.[8]

We therefore investigated the release of tryptase into the skin blister fluid in various dermatological disorders, especially urticaria.

Methods

Patients

A total of 48 patients were studied. Fifteen had chronic idiopathic urticaria (nine female and six male) with an age range of 19–70 years. All these were investigated as in-patients. Two patients had extensive urticaria pigmentosa and the remaining ones had a variety of disorders that included cholinergic urticaria, urticarial dermographism, angio-oedema, prurigo, psoriasis, hand eczema and atopic eczema. In addition, 10 healthy controls (five female and five male) with an age range of 23–31 years were studied.

Suction-blister technique

Suction blisters were produced by applying 350–400 mmHg suction to the skin for 60–75 min. Seven blisters, each 5 mm in diameter, were produced and when the blisters were fully formed the suction was discontinued. Blister fluid was immediately withdrawn using a sterile syringe over 1–2 min. The total volume of blister fluid was 100–200 μl which was placed in a plastic vial and immediately frozen in liquid nitrogen and kept at −80°C. None of the blisters contained erythrocytes in the fluid as judged by visual examination.

The suction blisters were induced on the volar aspects

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of forearms, abdomen, or thighs, at sites with urticarial lesions and in normal-looking skin in the patients with urticaria. Only the affected skin was studied in patients with mastocytosis. The remaining patients who suffered from eczema, prurigo of unknown origin, psoriasis, and atopic dermatitis, had blisters raised on normal-looking skin on the arms or abdomen. Suction blisters were also induced in patch-test areas or at tuberculin skin test sites on the forearms 0·5–3 h after applying the tests.

Blood samples were collected and after coagulation and centrifugation, the resulting serum was kept at −20°C until analysis.

Tryptase assay

Tryptase levels were analysed using a commercial assay (Pharmacia Tryptase RIACT, Pharmacia, Uppsala, Sweden). Briefly, the assay was a solid-phase radio immunoassay (RIA) based on two tryptase-specific monoclonal antibodies.[7] The tryptase in the sample (50 μl sample + 50 μl diluent) reacted with anti-tryptase antibody bound to the wall of the test tube, then ^{125}I-anti-tryptase (50 μl) was added to form a labelled complex. After overnight incubation at room temperature, the tubes were washed three times with 2 ml washing solution and the remaining radioactivity was determined. The data was evaluated against the tryptase standard samples (2, 5, 10, 20 and 50 μg/l) provided with the kit. The tryptase levels were expressed as μg/l. The intra-assay and inter-assay coefficients of variation were below 4% at 2 μg/l or higher tryptase concentrations.

Results

The suction-blister fluid in all the cases examined contained detectable levels of tryptase.

Chronic urticaria

Patients with chronic urticaria had significantly increased levels of tryptase in their skin compared to controls (Fig. 1). Normal-looking skin in patients with ongoing urticaria had lower levels of tryptase but these were still significantly higher than in controls. The normal-looking skin of one patient was studied 24 h after a severe urticarial attack, and high levels of tryptase were still present (> 50 μg/l). However, the level of tryptase declined in patients who had no skin lesions at the time of investigation, but who had urticaria some days previously. The level of tryptase was apparently not influenced by H1 blockers, because high levels of tryptase were found in patients on antihistamines but still with urticaria.

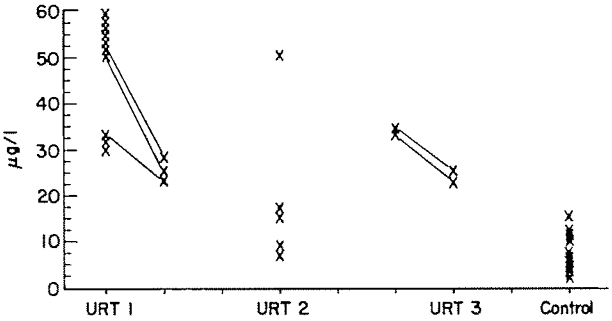

Figure 1. The figure shows individual levels of tryptase in suction-blister fluid from various patients with urticaria. URT1, suction-blister fluid taken from urticarial lesions in 10 patients. Three patients had suction blisters in clinically normal skin, but had active lesions elsewhere. URT2, five patients without clinical urticaria, but with recent urticarial eruption. URT3, two patients with urticaria pigmentosa, which were studied before and after 14 days of daily topical application of clobetasone dipropionate under 4-h occlusion. Control, 10 healthy controls.

Mastocytosis

Two patients with pronounced skin mastocytosis and without urticaria had increased levels of tryptase although not to the same extent as patients with active urticaria.

Cholinergic urticaria and urticarial dermographism

In one of two patients with cholinergic urticaria, and one patient with severe urticarial dermographism increased levels of tryptase were seen (results not shown).

Prurigo

Four patients complained of severe prurigo and were suspected of having chronic urticaria, but had no urticarial lesions (Fig. 2). One of these patients later developed disseminated lupus erythematosus and another neoplastic disease. One patient had previous angio-oedema, which was not present at the time of investigation.

Eczema

Five patients had various forms of eczema; two hyperkeratotic eczema of hands and feet, and three hand eczema

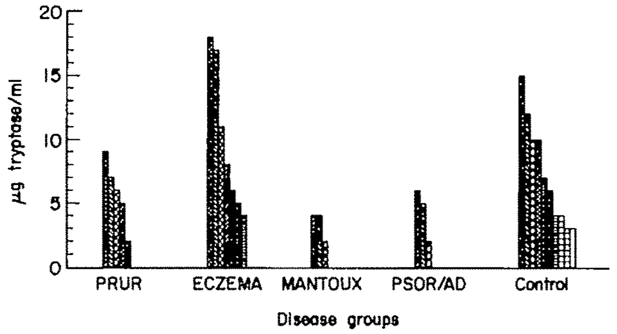

Figure 2. The figure shows individual levels of tryptase in suction-blister fluid from patients with a variety of skin disorders. PRUR, five patients suffering from prurigo of unknown cause, where urticaria was suspected but not observed. ECZEMA, five patients with contact allergies or keratotic eczema of hands. In two patients suction blisters were induced where a patch test was previously applied for 24 h. Control skin was also studied giving a total of seven measurements in this group. MANTOUX, one Mantoux-positive control had three suction blisters induced in normal skin and again 30 and 90 min following injection of tuberculin. No clinical reaction was observed. PSOR/AD, two patients with plaque psoriasis and one with active atopic dermatitis (AD) had suction blisters raised in normal skin (psoriasis) or in slightly eczematous skin (AD). Control, ten healthy controls.

and positive patch tests (nickel, fragrance mix, and formaldehyde), which were repeated on the volar aspect of their forearm in non-eczematous skin. Suction blisters were induced 30 min later before any clinical signs of an immune reaction. There was no significant increase in tryptase compared with controls (Fig. 2).

Psoriasis and atopic dermatitis

Two patients had psoriasis and the normal-looking skin close to a psoriatic lesion was studied; one had atopic dermatitis, and the suction blister was induced in normal-looking skin. In all these patients tryptase was found to be within the normal limits (Fig. 2)

Tuberculin skin tests

One healthy person had three tuberculin skin tests on the forearms using two units of tuberculin. Suction blisters were induced after 30 min and 2 h. Very low but detectable levels of tryptase were obtained (Fig. 2). There was an increase in tryptase after both 30 min and 2 h, but this was within the normal range and may have been a result of the intradermal injection.

Allergy to NCR paper

Four patients complained of itching and burning following contact with fresh NCR paper (no carbon copy paper). No differences in tryptase levels were found before and after exposure to NCR paper for 30 min. Three had normal levels of tryptase, whereas the fourth had significantly increased levels of tryptase (41 µg/l) both before and after contact with NCR paper. This patient had urticarial dermographism but not urticaria.

Serum levels of tryptase

Serum samples were taken from five patients with urticaria including those with skin mastocytosis. In none of the cases were increased levels of tryptase found (<2 µg/l) except in one of the patients with mastocytosis (2·1 µg/l). Serum was also investigated from two patients with severe atopic dermatitis, one patient with severe persistent light reactivity and one patient with severe erythroderma. Increased tryptase levels were not found (<2 µg/l) in any of the samples.

Discussion

This study found highly increased levels of tryptase in suction-blister fluid from patients with urticaria, being highest in fluid from active lesions, whereas tryptase could not be detected in the serum from these patients. This is probably due to the fact that the immunoreactivity of tryptase in serum is rapidly neutralized with a half-life of approximately 2 h[5], and suggests that the tryptase detected in skin originated from the skin mast cells through local release. It is highly unlikely that basophils contributed because they are not prominent in the skin of patients with urticaria and also contain very little tryptase.

Levels of tryptase were increased in clinically normal skin from patients with urticarial lesions elsewhere on the body or with recent urticaria and in patients with skin mastocytosis. A high level was also found in one patient with urticarial dermographism who complained of itching following contact with NCR paper. All other skin disorders including angio-oedema, prurigo, contact dermatitis, hyperkeratosis, eczema and psoriasis had tryptase levels within the range of the controls. Thus measurements of tryptase levels may help to distinguish cases of urticaria and to monitor activity of the disease. The mean level of tryptase in control skin found in this study (10 µg/l) corresponds well with data obtained by Shalit *et al.*[9] (7 µg/l).

It is possible that some of the released tryptase is removed or neutralized from the suction-blister fluid during the 60–75 min-period needed for blister induction. However, our observations show significant amounts of tryptase are present. This agrees with previous results showing an extended immunoreactivity *in vitro* of tryptase at 37°C (>20 h).[5]

It is not known to what extent the mechanical suction forces on skin may influence the release of tryptase from mast cells. However, they cannot explain the large differences in tryptase levels found in our study group. Another factor is the number of skin mast cells. This number was very high in one patient with mastocytosis, but we did not find a corresponding high level of tryptase in the suction-blister fluid from this patient. Also, we studied only volar skin, where the total amount of skin histamine is almost the same.[10] Tryptase release is apparently not related to the inflammatory response in the skin, because suction blisters were induced in normal-looking skin, apart from urticarial lesions. In one patient with atopic dermatitis the blisters were induced in an area of chronic eczema but increased levels of tryptase were not found.

Previous investigations have shown the release of histamine, prostaglandin D_2, leukotrienes C_4 and D_4 and tryptase during type I allergic reactions in the skin using skin windows[11–13] or suction blisters.[14–16] Tryptase has also been evaluated using biochemical and histochemical techniques in various human tissues.[17] The release of histamine and tryptase seems to correlate closely, although it was recently shown that ongoing antigen-stimulation leads to a continuous low but significant release of histamine lasting up to 6 h, whereas the level of tryptase falls to insignificant levels by that time.[9]

The present study is the first to demonstrate quantitatively the release of tryptase in the skin of patients with urticaria. It may provide an important technique for the evaluation of the participation of mast cells in skin inflammation. This technique also allows the *in-vivo* analysis of mast cell degranulation following injection of various mediators, and may provide a method for monitoring different treatments for urticaria and other mast-cell-dependent diseases and reactions.

References

1 Kaplan AP, Horakova Z, Katz SI. Assessment of tissue fluid histamine levels in patients with urticaria. *J Allergy Clin Immnol* 1978; 61: 350–4.

2 Misch KJ, Greaves MW, Kobza Black A. Histamine and the skin. *Br J Dermatol* 1983; 109 (Suppl. 24): 10–13.

3 Irani AA, Schechter NM, Craig SS *et al.* Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci (USA)* 1986; 83: 4464–8.

4 Schwartz LB, Irani A-MA, Roller K *et al.* Quantitation of histamine, tryptase and chymase in dispersed human T and TC mast cells. *J Immunol* 1987; 138: 2611–5.

5 Wenzel S, Irani A-MA, Sanders JM *et al.* Immunoassay of tryptase from human mast cells. *J Immunol Methods* 1986; 86: 139–42.

6 Schwartz LB, Bradford TR, Lee DC, Chlebowski JF. Immunologic and physiological evidence for conformational changes occurring on conversion of human mast cell tryptase from active tetramer to inactive monomer. Production of monoclonal antibodies recognizing active tryptase. *J Immunol* 1990; 144: 2304–11.

7 Enander I, Matsson P, Nystrand J *et al.* A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. *J Immunol Methods* 1991; 138: 39–46.

8 Schwartz LB, Metcalfe DD, Miller JS *et al.* Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987; 316: 1622–6.

9 Shalit M, Schwartz LB, Golzar N *et al.* Release of histamine and tryptase *in vivo* after prolonged cutaneous challenge with allergen in humans. *J Immunol* 1988; 141: 821–6.

10 Eady RAJ, Cowen T, Marshall TF *et al.* Mast cell population density, blood vessel density and histamine content in normal human skin. *Br J Dermatol* 1979; 100: 623–33.

11 Dunsky EH, Zweiman B. The direct demonstration of histamine release in allergic reactions in the skin using a skin chamber technique. *J Allergy Clin Immunol* 1978; 62: 127–30.

12 Ting S, Dunsky EH, Lavker RM, Zweiman B. Patterns of mast cell alterations and *in vivo* mediator release in human allergic reactions. *J Allergy Clin Immunol* 1980; 66: 417–23.

13 Pienkowski MM, Adkinson NF, Plaut M *et al.* Prostaglandin D_2 and histamine during the immediate and the late-phase components of allergic cutaneous reactions. *J Allergy Clin Immunol* 1988; 82: 95–100.

14 Dorsch W, Ring J. Induction of late cutaneous reactions by skin blister fluid from allergen tested and normal skin. *J Allergy Clin Immunol* 1981; 67: 117–23.

15 Dorsch W, Ring J, Reimann HJ, Geiger R. Mediator studies in skin blister fluid from patients with dual reactions after intradermal allergen injection. *J Allergy Clin Immunol* 1982; 70: 236–42.

16 Dorsch W, Ring J, Weber PC, Strasser T. Detection of immunoreactive leucotrienes LTC4/LTD4 in skin blister fluid after allergen challenge in patients with the late cutaneous reactions. *Arch Dermatol Res* 1985; 277: 400–1.

17 Harvima IT, Karkola K, Harvima RJ *et al.* Biochemical and histochemical evaluation of tryptase in various human tissues. *Arch Dermatol Res* 1989; 281: 231–7.

British Journal of Dermatology (1991) **125**, 18–20. ADONIS 000709639100157Z

In-vivo effects of solar-simulated ultraviolet irradiation on antioxidant enzymes and lipid peroxidation in human epidermis

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Accepted for publication 30 January 1991

Summary The effects of solar-simulated UV-irradiation on the activity of antioxidant enzymes and the amount of diene conjugation were studied in human epidermis *in vivo*. A single dose of UV-irradiation was found to result in a transient reduction in superoxide dismutase activity and this was followed by increased amounts of conjugated diene double bonds, an index for oxidative stress. This suggests that *in-vivo* exposure of human epidermis to solar-simulated UV-irradiation causes changes in the enzymic antioxidant defence system which, in turn, are accompanied by increased level of oxidative stress.

Chronic exposure to sunlight is known to result in an increased risk of cutaneous malignancies.[1] The cellular pro-oxidant states, defined as increased concentrations of active oxygen species, organic peroxides and radicals, are implicated in a number of physiological and pathological processes, including both cellular ageing and chemical carcinogenesis.[2,3] A pro-oxidant state may be due to either over-production of the reactive substances or a deficiency in the antioxidant defence system. Recent studies indicate that an exposure to UVB-irradiation may result in impairment of the antioxidant defence system in experimental animals as well as in tissue and cell cultures.[4–8] In this study we have investigated the effects of solar-stimulated UV-irradiation on human epidermis *in vivo*.

Methods

Epidermis samples and UV exposures

Fourteen male volunteers (aged 18–22 years) were exposed to one dose of solar-simulated UV-irradiation corresponding to 2 MED (minimal erythema dose). The light source was a UVB Osram HQI-TS 400 W/Berger Xenon solar simulator, with a radiation spectrum of 285–350 nm and a peak at 315 nm. The light source irradiated in the UVB range (280–320 nm) at 1·9 mW/cm^2, and in the UVA range (320–400 nm) at 25 mW/cm^2. The UV doses were measured using a Waldmann UV-meter. The average MED, measured as UVB-irradiation, was 50 mJ/cm^2. Both control epidermis and UV-irradiated epidermis were obtained using the suction-blister technique (suction for 2 h)[9] within 5 min of UVB irradiation, at 3 h, and at 24 h post-irradiation. The samples were then frozen at -20°C and analysed within 4 weeks.

Enzyme activities and percentage of conjugated diene double bonds.

The percentage of conjugated diene double bonds as well as the analysis of glutathione peroxidase (with cumene hydroperoxide as substrate) and glutathione transferase activity were performed using previously described methods[10–12]. Catalase activity was determined by measuring the rate of disappearance of 15 mM hydrogen peroxide at 240 nm.[13] Superoxide dismutase (Cu/Zn) was assayed spectrophotometrically by inhibition of epinephrine auto-oxidation.[14] Standard curves for the enzyme activities of catalase and superoxide dismutase were made by using purified enzyme preparations. Enzyme activities were expressed as μg of the purified enzyme preparation/mg protein in the cell homogenate. In the analysis of catalase activity, 1 μg of the enzyme preparation (bovine liver catalase; E.C. 1.11.1.6; Sigma) corresponded to 2·5 U, and 1 μg of superoxide dismutase preparation (bovine erythrocytes; E.C. 1.15.1.13; Sigma) corresponded to 3·6 U.

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Table 1. The activities of antioxidant enzymes in human epidermis following exposure to solar-simulated UV-irradiation

	SOD	CATALASE	GSH-PX	GSH-TF
Controls	100	100	100	100
Immediate	62±11** $n=6$	182±34* $n=6$	100±20 $n=6$	85±18 $n=6$
3 h	102±6 $n=8$	113±16 $n=8$	97±3 $n=7$	134±23* $n=6$
24 h	116±11 $n=8$	90±17 $n=7$	95±6 $n=8$	126±23 $n=7$

The enzyme activities are expressed as the percentage of the time-matched control value (mean ± SEM), and at the different time points the statistical significances between the irradiated and control epidermis were calculated using Students *t*-test for paired data: * $=P<0\cdot05$, ** $=P<0\cdot01$. The control groups at the different time points did not significantly differ from each other, and the mean ± SEM values of the control samples correspond to the following activities: superoxide dismutase (SOD) 7·5±0·5 units/mg protein ($n=22$); catalase 2·8±0·2 units/mg protein ($n=21$); glutathione peroxidase (GSH-PX) 20±0·6 nmol/min/mg protein ($n=21$); glutathione transferase (GSH-TF) 32±2·5 nmol/min/mg protein ($n=19$).

Results

The activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione transferase at the different time points are shown in Table 1. As measured immediately after the UV-irradiation, the activity of Cu/Zn-superoxide dismutase (SOD) was found to be significantly decreased, while catalase activity was substantially increased. At 3 h post-irradiation, glutathione transferase activity was slightly increased, while the glutathione peroxidase activity was unchanged at all time points. The percentage of conjugated diene double bonds was 88±8%, 259±55% ($P<0\cdot05$), and 147±24% ($P<0\cdot05$) of the control value as measured immediately, 3 h, and 24 h post-irradiation, respectively (Table 1).

Discussion

Superoxide dismutase activity was found to be decreased immediately after solar-simulated UV-irradiation, and UVB radiation (280–320 nm) can be considered mainly responsible for this decrease. The magnitude of the change can be considered biologically significant because it is known from genetic disorders that even small changes in the amount of superoxide dismutase can have important effects on the cellular responses to oxidative stress.[15] Consistent with this, after the decrease in superoxide dismutase activity the level of oxidative stress, as measured by the percentage of conjugated diene double bonds, was found to be increased. The present results show that the amount of lipid peroxidation is increased following solar-simulated UV-irradiation, not only in cell cultures but also *in vivo* in human skin. The decrease in SOD activity may be due either to inactivation of the enzyme by the hydrogen peroxide formed,[16] or to the direct action of UV-irradiation. Prolonged exposure to reactive oxygen species results in induction of pulmonary antioxidant enzymes,[17] and it remains to be investigated whether similar compensatory mechanisms are functioning in skin, e.g. following UVB phototherapy. The observed decrease in superoxide dismutase activity is consistent with earlier studies of both murine skin and cultured human keratinocytes,[5–7] but the increase in catalase activity following UV-irradiation contrasts with recent studies on cell and tissue cultures.[6,7] Catalase activity, however, has been suggested to be of minor importance in the protection of cells from lipid peroxidation, because it is known that glutathione peroxidase (Se-enzyme) removes most if not all of the hydrogen peroxide originating from the endoplasmic reticulum.[18]

In conclusion, this experimental model provided a means of investigating the biochemistry of human epidermis *in vivo*. The present results demonstrate that an *in-vivo* exposure of human epidermis to solar-simulated UV-irradiation is followed by impaired detoxification of reactive oxygen and elevated oxidative stress, which, in turn, have been shown to be associated with chemical carcinogenesis and tumour promotion.[2] It remains to be elucidated to what extent this mechanism of action of UV-irradiation contributes to the carcinogenic and tumorigenic properties of solar light and UVB radiation.

Acknowledgments

The technical assistance of Mrs Raija Soderholm and Mrs Leena Soderholm is gratefully acknowledged. The present study was financially supported by The Academy of Finland, The Juho Vainio Foundation, The Research and Science Foundation of Farmor Ltd, and the Finnish Cancer Foundation.

References

1 MacKie RM, Rycroft MJ. Health and the ozone layer. *Br Med J* 1988; **297**: 369–70.
2 Cerutti PA. Prooxidant states and tumor promotion *Science* 1985; **227**: 375–81.

3 Harman D. Free radical theory of aging. *Age* 1984; **7:** 111–31.

4 Miyachi Y, Imamura S, Niwa Y. Decreased skin superoxide dismutase activity by a single exposure of ultraviolet radiation is reduced by liposimal superoxide dismutase pretreatment. *J Invest Dermatol* 1987; **89:** 111–2.

5 Miyachi Y, Imamura S. Photo-oxidative skin damage and antioxidants. *Photodermal Photoimmunol Photomed* 1990; **7:** 49–50.

6 Fuchs J, Huflejt M, Rothfuss L *et al.* Impairment of enzymic and nonenzymic antioxidants in skin by UVB irradiation. *J Invest Dermatol* 1989; **93:** 769–73.

7 Punnonen K, Puntala A, Jansén CT, Ahotupa M. UVB irradiation induces lipid peroxidation and decreases antioxidant enzyme activities in human keratinocytes *in vitro*. *Acta Derm Venereol (Stockh)* 1991; (in press).

8 Punnonen K, Jansén CT, Puntala A, Ahotupa M. Effects of *in vitro* UVA irradiation and PUVA treatment on membrane fatty acid and activities of the antioxidant enzymes in human keratinocytes. *J Invest Dermatol* 1991; **96:** 255–9.

9 Kiistala U, Mustakallio KK. *In vivo* separation of epidermis by preparation of suction blisters. *Lancet* 1964; **i:** 1444.

10 Ahotupa M, Bussacchini-Griot V, Bereziat J-C *et al.* Rapid oxidative stress induced by N-nitrosoamines. *Biochem Biophys Res Commun* 1987; **146:** 1047–54.

11 Paglia D, Valentine W. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70:** 158–69.

12 Habig WH, Pabst MJ, Jacoby WB. Glutathione S-transferase. The first enzymic step in mercapturic acid formation. *J Biol Chem* 1974; **249:** 7130–9.

13 Beers B, Sizer W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; **195:** 133–9.

14 Misra HP, Fridovich J. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247:** 3170–5.

15 Ackerman A, Fackler J, Tuck-Muller CM *et al.* Partial monosomy 21, diminished activity of superoxide dismutase, and pulmonary oxygen toxicity. *N Engl J Med* 1988; **318:** 1666–9.

16 Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme. *Biochemistry* 1975; **14:** 299.

17 Hoffman M, Stevens JB, Autor AP. Adaptation to hyperoxia in the neonatal rat: kinetic parameters of the oxygen mediated induction of lung superoxide dismutases, catalase and glutathione peroxidase. *Toxicology* 1980; **16:** 215–25.

18 Orrenius S, Thor H, Eklöw L *et al.* Drug-stimulated H_2O_2 formation in hepatocytes. Possible toxicological implications. *Adv Exp Med Biol* 1981; **136 (A):** 395–405.

British Journal of Dermatology (1991) **125**, 21–26.

ADONIS 000709639100158E

The epidermal basement membrane in basal cell carcinoma: an immunohistochemical study

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Accepted for publication 2 February 1991

Summary An immunohistochemical study of basal cell carcinomas of varying histological type, using a panel of antibodies to constituents of the epidermal basement membrane, showed marked deficiencies in the expression of the antigens identified by the antibodies LH7·2, GB3 and G71. There was no correlation between loss of immunoreactivity to these antibodies and the histological features of the tumour.

The precise mechanisms by which malignant keratinocytes penetrate stromal tissue and breach endothelial basement membrane in the process of metastatic spread are not clearly established, but the potential importance of physical containment of tumour cells by an intact epidermal basement membrane (EBM) has been emphasized.[1] Breaches of basement membrane are widely described in malignant but not benign tumours of many organs,[2] although abnormalities in the structural integrity of basement membrane are not restricted to malignancy, being found in a variety of inflammatory disorders.[3]

One theory of metastasis is that transformed epidermal cells produce degradative enzymes that are substrate-specific for basement membrane components and connective tissue stroma which facilitates invasion. The *in-vitro* and *in-situ* demonstration of collagenase around basal cell carcinomas (BCC)[4,5] suggest the potential for dissolution of the collagenous components of the EBM during invasion, as type IV collagen constitutes the major structural protein of the EBM[6] and type VII collagen is the major constituent of anchoring fibrils.[7]

The recent development of highly specific antibodies to ultrastructurally defined epitopes within the several strata of the dermo-epidermal junction now allow a more detailed *in-situ* study of individual antigenic constituents of the EBM around cutaneous malignancies. The invasive characteristics of BCC, consisting of progressive, occasionally aggressive, local spread with a very low propensity to metastasize, makes this tumour of particular interest with regard to the biological role played by EBM in the spread of malignant keratinocytes.

Methods

Tissue samples

Elliptical excision was performed on 33 cases of BCC under local anaesthesia using 1% lignocaine alone, infiltrated around the lesion. All patients gave informed consent. The specimens were bisected with one half undergoing routine paraffin processing for histological confirmation and classification, while the other half was frozen in liquid nitrogen-cooled isopentane using OCT cryoprotectant (Tissue Tec, Miles, U.S.A.) and stored at − 70°C until used. The 33 cases of BCC used in this study consisted of nodular (15), cystic (5), morphoeic (7) and superficial (6) histological patterns. Normal, non-lesional hair-bearing skin (scalp three, face two) was used as control tissue to confirm the reactivity of the antibodies to appendageal structures and blood vessels in addition to interfollicular EBM.

Primary antibodies used

The monoclonal antibody (MAb) LH7·2 recognizes an epitope on the carboxyl terminus of type VII collagen, the major component of anchoring fibrils (AF), at the point of insertion of AF into the lamina densa.[7] A MAb to type IV collagen, a protein thought to be crucial to the structural integrity of the EBM, is localized at the lamina

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Table 1. Details of primary antibodies used

Antibody	Dilution	Reactivity	Source
LH7 2	1:10	Type VII collagen (anchoring fibrils)	Dr I.M.Leigh*
Anti-type IV	1:50	Type IV collagen (lamina densa)	Dr I.M.Leigh*
Anti-laminin	1:50	Laminin (lamina lucida)	E.Y. Labs Inc.†
GB3	1:50	Hemidesmosome associated proteins (lamina lucida)	Sera Lab. Ltd‡
G71	1:10	Glycoprotein complex (lamina lucida)	Dr J.D.Aplin§

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densa.[6] The lamina lucida contains the antigens defined by a polyclonal antibody to laminin, a non-collagenous glycoprotein common to all basement membranes;[8] GB3, a MAb raised against an extract of human amnion which recognizes a non-collagenous protein constituted from five polypeptides, which localizes within the lamina lucida in close association with overlying hemidesmosomes;[9] and G71, a MAb raised against human endometrial epithelial cells which recognizes glycoproteins within the lamina lucida closely associated with the extracellular aspect of the basal epithelial cell.[10]

Immunohistochemistry

Serial 5-μm cryostat sections were mounted on glass slides, air dried and fixed in acetone prior to performing a standard indirect immunoperoxidase staining technique.[11] Briefly, sections were incubated with primary antibodies for 60 min (see Table 1 for dilutions used), followed by peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako U.K., High Wycombe, Bucks, U.K.) for 30 min. Visualization was accomplished using 3,3′-diaminobenzidine (DAB) as the substrate for peroxidase for 10 min. All stages of the immunohistochemical reaction were separated by 10-min washes with Tris-buffered saline. Slides were counterstained using Mayers haemalum, dehydrated and cleared in xylene prior to mounting in DPX. Normal skin and perilesional skin were used as positive controls; negative controls consisted of omission of primary antibodies, their substitution by irrelevant MAbs of similar isotype, omission of secondary antibodies and the use of DAB alone.

Results

Normal skin

Antibodies to type IV collagen and laminin stained all basement membranes present in tissue sections; endothelial, smooth muscle and neuronal basement membranes were also demonstrated. Staining for type VII collagen and GB3 was, however, limited to the epidermal basement membrane. The G71 antibody gave continuous staining of the EBM and produced weak staining of some dermal endothelial BMs and some cytoplasmic staining of basal keratinocytes. All antibodies gave uninterrupted staining of the hair follicle apparatus, similar to that seen in interfollicular epidermis.

Basal cell carcinoma

All 33 cases of BCC showed continuous linear staining of the EBM around tumour islands for type IV collagen and laminin (Figs 1 and 2). Whilst the intensity of staining for type IV collagen in six cases (18%) and for laminin in 12 cases (36%) did show some quantitative reduction in focal staining compared with normal EBM, no areas without staining were noted, even in areas adjacent to dense mononuclear infiltration. There was no apparent correlation between the histological pattern of BCC and the staining pattern.

In contrast to type IV collagen, staining for type VII collagen was completely absent in 24 of 33 cases (73%). An abrupt transition was seen between the normal interfollicular BM and the tumour islands (Fig. 1). In the remaining nine cases, pronounced diminution of stain-

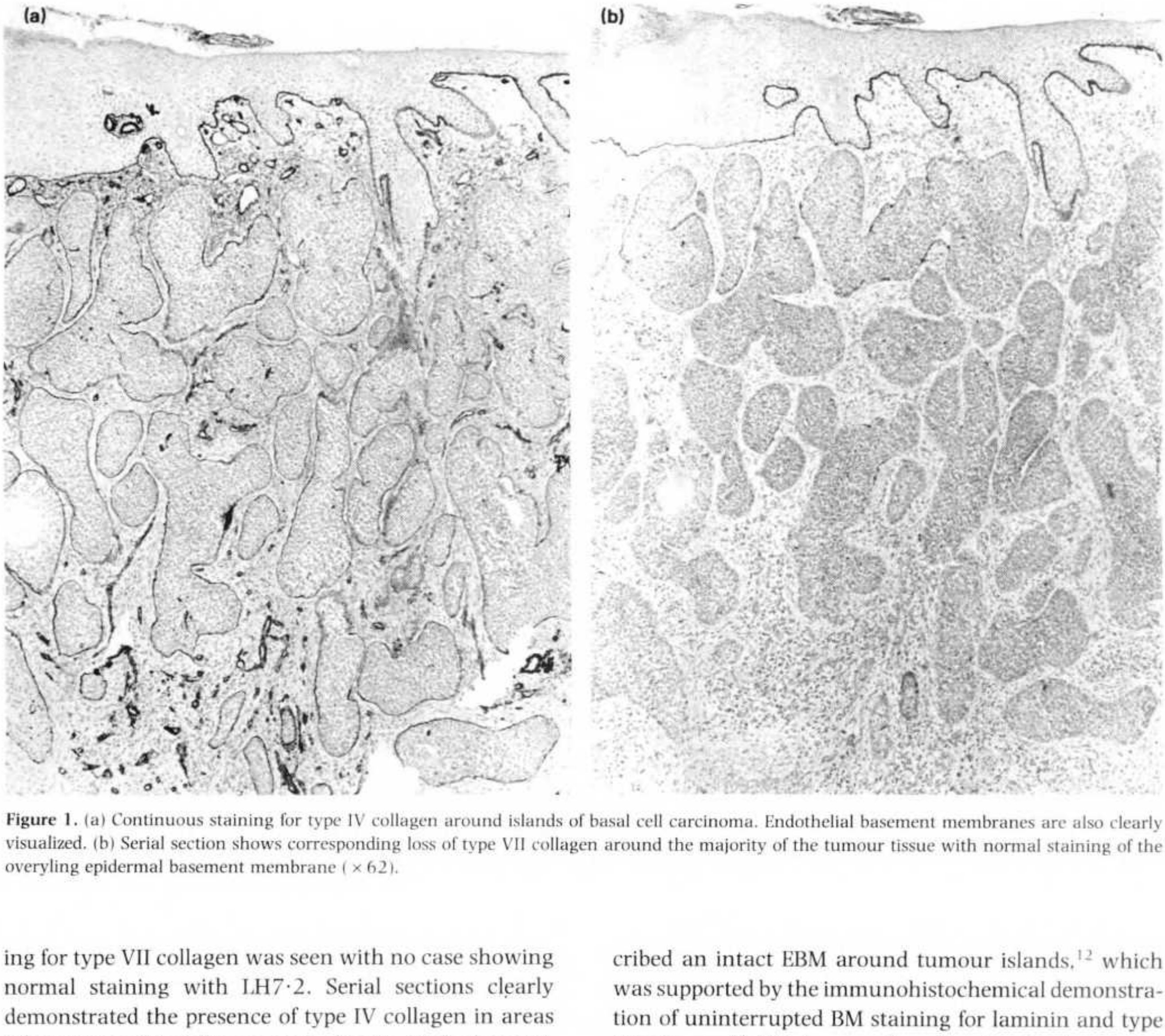

Figure 1. (a) Continuous staining for type IV collagen around islands of basal cell carcinoma. Endothelial basement membranes are also clearly visualized. (b) Serial section shows corresponding loss of type VII collagen around the majority of the tumour tissue with normal staining of the overyling epidermal basement membrane (× 62).

ing for type VII collagen was seen with no case showing normal staining with LH7·2. Serial sections clearly demonstrated the presence of type IV collagen in areas where type VII collagen was absent, with the peri-tumoural EBM acting as a positive internal control for type VII collagen.

Staining for the lamina lucida antigens recognized by GB3 and G71 also showed widespread abnormalities, frequently mirroring the losses of type VII collagen. GB3 staining was lost in 30 of 33 cases (91%) (Fig. 3). The remaining three cases showed a marked reduction in the intensity of staining with numerous foci of attenuation. Similarly, G71 staining was totally absent or markedly reduced around the tumour islands of all BCC studied (Fig. 4).

Discussion

Electron microscopic (EM) studies of BCC initially described an intact EBM around tumour islands,[12] which was supported by the immunohistochemical demonstration of uninterrupted BM staining for laminin and type IV collagen.[13–16] Subsequently, ultrastructural morphological studies of the stroma around islands of BCC have demonstrated irregularities in the EBM[17–22] and widespread degenerative changes of the stromal collagen adjacent to the tumour cells.[19] Immunohistochemical studies have demonstrated a defect in staining for the bullous pemphigoid antigen (BPA)[13,18,23–25] and anchoring fibril antigens AF1 and AF2.[25] Our findings confirm the earlier reports of continuous EBM staining for laminin and type IV collagen but demonstrate widespread absence of the GB3 and G71 epitopes from the lamina lucida, and the reduction of the anchoring fibril component type VII collagen. These findings for type VII collagen are at variance with those reported recently using a different antibody.[20] The results of our study suggest that the loss of BPA is not an isolated defect but

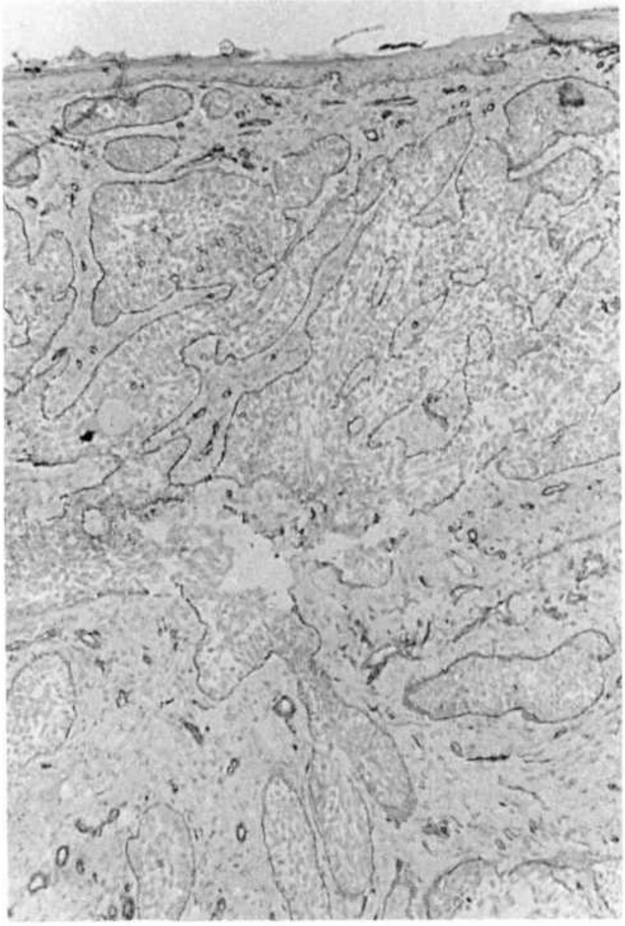

Figure 2. Uninterrupted staining for laminin around islands of basal cell carcinoma (× 80).

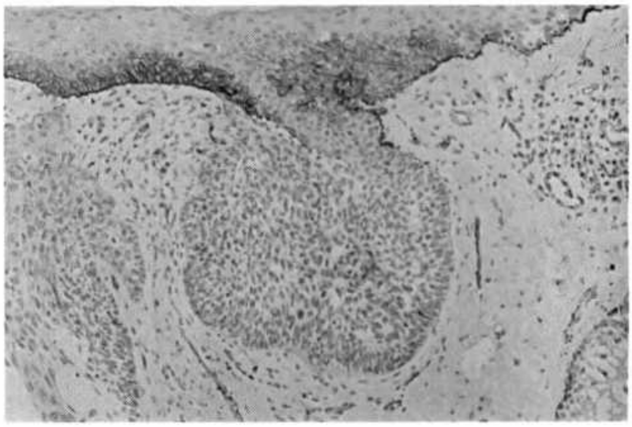

Figure 4. Loss of staining for G71 around a superficial island of BCC. Note the staining of endothelial BM and cytoplasmic staining of some basal keratinocytes (× 70).

part of a more profound antigenic alteration in the EBM around BCC.

The histological and clinical variants of BCC—superficial, nodulocystic, pigmented and morphoeic[26]—have a variable capacity for local invasion. The morphoeic form is considered to have the greatest invasive potential. We failed, however, to demonstrate any consistent correlation between the antigenic integrity of the EBM and the particular histological type of BCC, and are unable to support reports of the loss of type IV collagen and laminin from around morphoeic BCC.[27,28] Small buds of multifocal BCC arising from the epidermis

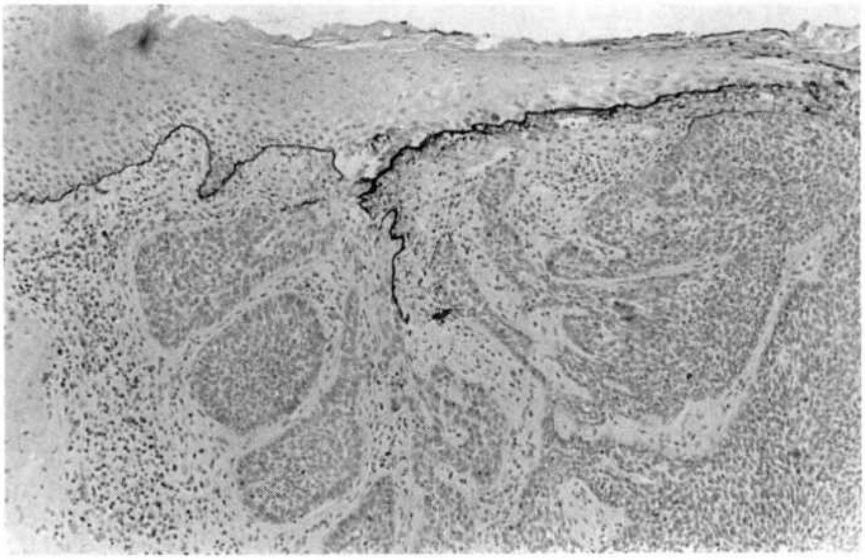

Figure 3. Abrupt loss of GB3 staining on entering basal cell carcinoma (× 84).

frequently showed identical staining reactions to deep, locally invasive morphoeic lesions.

Perhaps the most significant finding of this study is the demonstration of a difference in type IV and type VII collagen expression around BCC. Preliminary data from other sources is in agreement with these findings.[29] One of the theories as to how tumour cells invade local tissue concerns the production of degradative enzymes, specific for BM and surrounding connective tissue components. Neoplastic cells,[30] including BCC[4,28] produce collagenases which possess activity against collagen components in the EBM and surrounding stroma.[31] Elevated levels of human skin collagenase, normally produced by fibroblasts, have been immunolocalized to the stromal tissue immediately adjacent to BCC tumour islands but not to the tumour cells themselves.[5] Recent work suggests that human skin collagenase is able to degrade type VII but not type IV collagen.[32] The production of such a collagenase by stromal cells, perhaps in response to factors released by tumour cells, would lead to a selective dissolution of type VII collagen consistent with the results of this study, without substantially impairing the structural integrity of type IV collagen. Loss of anchoring fibrils and stromal collagen may be important factors in allowing local invasion or expansion of BCC within a still intact type IV collagen membrane.

A putative role for type IV collagen in containment of malignant cells is supported by the demonstration that tumour cell lines with the greatest metastatic potential[33] and areas of invasive breast carcinoma[34] produce a different collagenase with type IV collagen degrading activity. Studies addressing type IV collagen staining patterns in epidermal tumours which frequently metastasise, such as squamous cell carcinoma, may further clarify the role of type IV collagen in cutaneous malignancies.

Whilst diminution of immunoreactivity may represent the true loss of a molecule due to enhanced catabolism via enzymatic degradation, other possibilities such as impaired production, assembly or secretion of BM components by tumour cells cannot be excluded. It is clearly established that epithelial cells in culture manufacture BM components,[35–37] and the loss of staining of certain constituents noted in this study may, therefore, represent an impaired ability of the neoplastic cells to manufacture the normal repertoire of BM components. A further explanation for the absence of EBM antigens around BCC would be that the cell type of origin (possibly a subpopulation of appendageal basal cells) does not have the phenotypic potential to produce type VII collagen or the G71 and GB3 antigens. However, our own observations do not support this as all the EBM antigens examined gave continuous staining along the entire hair follicle structure, as well as the interfollicular regions.

This study has demonstrated widespread deficiencies in the antigenic structure of the EBM around BCC, although the relationship between these antigenic abnormalities and the invasive potential of the tumour remains uncertain.

References

1 Vracko R. Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. *Am J Path* 1974; **77:** 314–46.

2 Barsky SH, Siegal GP, Jannotta F, Liotta LA. Loss of basement membrane components by invasive tumours but not by their benign counterparts. *Lab Invest* 1983; **49:** 140–7.

3 Heng MCY, Kloss SG, Kuehn CS, Chase DG. Significance and pathogenesis of basal keratinocyte herniations in psoriasis. *J Invest Dermatol* 1986; **87:** 362–6.

4 Yamanishi Y, Dabbous MK, Hashimoto K. Effect of collagenolytic activity in basal cell epithelioma of the skin on reconstituted collagen and physical properties and kinetics of the crude enzyme. *Cancer Res* 1972; **32:** 2551–60.

5 Bauer EA, Gordon JM, Reddick ME, Eisen AZ. Quantitation and immunocytochemical localization of human skin collagenase in basal cell carcinoma. *J Invest Dermatol* 1977; **69:** 363–7.

6 Briggaman RA. Biochemical composition of the epidermo-dermal junction and other basement membrane. *J Invest Dermatol* 1982; **78:** 1–6.

7 Leigh IM, Eady RAJ, Heagerty AHM *et al.* Type VII collagen is a normal component of epidermal basement membrane, which shows altered expression in recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 1988; **90:** 639–42.

8 Foidart JM, Bere EW, Yaar M *et al.* Distribution and immunoelectron microscopic localization of laminin, a non collagenous basement membrane glycoprotein. *Lab Invest* 1980; **42:** 336–42

9 Verrando P, Hsi B-L, Yeh C-J *et al.* Monoclonal antibody GB3, a new probe for the study of human basement membranes and hemidesmosomes. *Exp Cell Res* 1987; **170:** 116–28.

10 Aplin JD, Self MW. Basally located epithelial cell surface component identified by a novel monoclonal antibody technique. *Exp Cell Res* 1985; **160:** 550–5.

11 Holden CA, MacDonald DM. Immunoperoxidase techniques in dermatopathology. *Clin Exp Derm* 1983; **8:** 433–57.

12 Zelickson AS. Electron microscope study of the basal cell epithelioma. *J Invest Dermatol* 1962; **39:** 183–7.

13 Stanley JR, Beckwith JB, Fuller RP, Katz SI. A specific antigenic defect of the basement membrane is found in basal cell carcinoma but not in other epidermal tumours *Cancer* 1982; **50:** 1486–90.

14 Van Cauwenberge D, Pierard GE, Foidart JM, Lapiere ChM. Immunohistochemical localization of laminin, type IV and type V collagen in basal cell carcinoma. *Br J Dermatol* 1983; **108:** 163–70.

15 Nelson DL, Little CD, Balian G. Distribution of fibronectin and laminin in basal cell epitheliomas. *J Invest Dermatol* 1983; **80:** 446–52.

16 Weber L, Krieg T, Muller PK *et al.* Immunofluorescent localization of type IV collagen and laminin in human skin and its application in junctional zone pathology. *Br J Dermatol* 1982; **106:** 267–73.

17 Pierard GE, Hermanns JF, Lapiere ChM. Stereologie de l'interface dermo-epidermique. *Dermatologica* 1974; **149**: 266–73.

18 Kimura S, Yamasaki Y, Nishiakowa T *et al.* A comparative study of seborrhoeic keratoses and basal cell epitheliomas by complement immunofluorescence and electron microscopy. *Acta Derm Venereol (Stockh)* 1980; **60**: 203–7.

19 Hashimoto K, Yamanishi Y, Dabbous MK. Electron microscopic observations of collagenolytic activity of basal cell epithelioma of the skin *in vivo* and *in vitro*. *Cancer Res* 1972; **32**: 2561–7.

20 Jones JCR, Steinman HK, Goldsmith BA. Hemidesmosomes, collagen VII and intermediate filaments in basal cell carcinoma. *J Invest Dermatol* 1989; **93**: 662–71.

21 Kobayasi T. Dermoepidermal junction in basal cell carcinoma. *Acta Derm Venereol (Stockh)* 1970; **50**: 401–11.

22 McNutt NS. Ultrastructural comparison of the interface between epithelium and stroma in basal cell carcinoma and control human skin. *Lab Invest* 1976; **35**: 132–42.

23 de Moragas JM, Winkelmann RK, Jordon RE Immunofluorescence of epithelial skin tumours. *Cancer* 1970; **25**: 1404–7.

24 Tosca A, Varelzidis A, Nicolis G *et al.* Antigenic alterations in tumours of epidermal origin. *Cancer* 1980; **45**: 2284–90.

25 Lane AT, Goldsmith LA, McCoon PE, Muhlbauer JE. Decreased anchoring-fibril antigens (AF1 and AF2) in basal-cell carcinoma. *Arch Dermatol Res* 1985; **277**: 499–501.

26 McGibbon DJ. Malignant epidermal tumours. *J Cutan Pathol* 1985; **12**: 224–33.

27 Kallioinen M, Antio-Harmainen H, Damment K *et al.* Discontinuity of the basement membrane in fibrosing basocellular carcinomas and basosquamous carcinomas of the skin: an immunohistochemical study with human laminin and type IV collagen antibodies. *J Invest Dermatol* 1984; **82**: 248–51.

28 Barsky SH, Grossman DA, Bhuta S. Desmoplastic basal cell carcinomas possess unique basement membrane-degrading properties. *J Invest Dermatol* 1987; **88**: 324–9.

29 d'Ardenne AJ, Butler MG, Andrews J *et al.* Type VII collagen in basal cell carcinoma. *J Pathol* 1987; **151**: 95–6A.

30 Dresden MH, Heilman SA, Schmidt JD. Collagenolytic enzymes in human neoplasms. *Cancer Res* 1972; **32**: 993–6.

31 Liotta LA, Kleinerman J, Cantanzaro P, Rynbrandt D. Degradation of basement membrane by murine tumour cells. *J Natl Cancer Inst* 1977; **58**: 1427–31.

32 Seltzer JL, Eisen AZ, Bauer EA *et al.* Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin. *J Biol Chem* 1989; **264**: 3822–6.

33 Liotta LA, Abe S, Robey PG, Martin GR. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumour. *Proc Natl Acad Sci USA* 1979; **76**: 2268–72.

34 Barsky SH, Togo S, Garbisa S, Liotta LA. Type IV collagenase immunoreactivity in invasive breast carcinoma. *Lancet* 1983; **i**: 296–7.

35 Alitalo K, Keski-Ofa J, Vaheri A. Extracellular matrix proteins characterise human tumour cell lines. *Int J Cancer* 1981; **27**: 755–61.

36 Hirone T, Taniguchi S. Basal lamina formation by epidermal cells in cell culture. In *Current Problems in Dermatology* (Bernstein AI, Seiji M, eds), Vol 10. New York: S Karger AG, 1980; 159–69.

37 Saki LY, Keene DR, Morris NP, Burgeson RE. Type VII collagen is a major structural component of anchoring fibrils. *J Cell Biol* 1986; **103**: 1577–86.

British Journal of Dermatology (1991) 125, 27–29. ADONIS 000709639100159R

Nail is produced by the normal nail bed: a controversy resolved

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Accepted for publication 28 January 1991

Summary

Nail thickness and mass (dry weight/unit surface area) of 21 toenails, removed from 19 patients after accidental injury, were measured over the mid point of the lunula, at the nail plate immediately distal to the lunula and at the distal end of the nail bed. Nail thickness increased from 43% of the final thickness over the mid-point of the lunula to 81% at its distal margin, the remaining increase in thickness being formed by the nail bed. The changes in nail mass were comparable. We conclude that ventral nail produced by the nail bed comprises about one-fifth of the terminal nail thickness and mass.

There are conflicting views as to whether the nail is formed only by the matrix of the lunula or whether the nail bed contributes as the nail plate grows out.[1–14] If the nail bed does contribute to the nail plate, then the nail would progressively increase in thickness and mass as it moves over the nail bed; we therefore measured nail thickness and mass at three defined sites along its length.

Methods

Clinically normal human nails, removed from the big toes of normal subjects because of accidental injury, were studied. A total of 21 nails, removed from 14 males (mean age 29 years, range 14–67 years) and five females (mean age 27 years, range 12–40 years), were immediately placed in airtight screw-cap containers and processed as soon as possible in the laboratory. Each nail was wiped with gauze, eponychial tissue at the germinal matrix was removed with dissecting forceps, then the nail was dried to constant weight for 3 days in a vacuum oven.

Nail thickness

Nail thickness was measured at (i) the mid-point of the lunula, between the proximal margin of the avulsed nail and the distal end of the lunula, and corresponding to the germinal matrix; (ii) the proximal nail, 1 mm distal to the lunula and corresponding to the proximal margin of the nail bed; (iii) the distal nail, at a point proximal to the free edge of the nail and corresponding to the most distal part of the nail bed at the beginning of the onychodermal band (Fig. 1a). Measurements were made with a screw-gauge micrometer or, when curvature prevented its use, with a vernier calliper. The thickness of each sample was calculated from the mean of three measurements with a SE of < ±7%.

Nail mass

After nail thickness was measured, each nail was cut with a scalpel in a line parallel to the hyponychium to give three samples corresponding to the three areas under study (Fig. 1b). The outline of each sample was traced onto paper and the surface area of each measured by planimeter. The mean for each of the three areas was calculated from 10 complete tracings of each nail sample and gave a SE < ±5%. Only 19 of the 21 nails were used

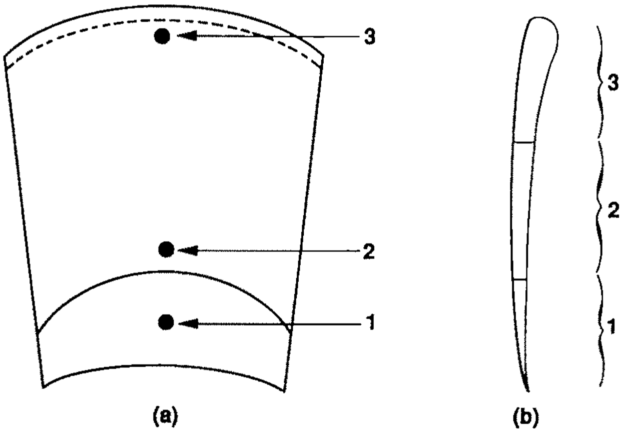

Figure 1. (a) Points at which nail thickness was measured. 1 = mid-point of lunula (germinal matrix); 2 = proximal margin of nail bed (1 mm distal to lunula); 3 = distal margin of nail bed just proximal to onychodermal band. (b) Sites of separation of nail into three zones for measurement of mass. 1 = lunula; 2 = proximal half nail plate beyond lunula; 3 = distal half of nail plate beyond lunula.

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because two fragmented whilst being cut. The samples were weighed by a balance measuring to an accuracy of 1×10^{-6} g and the mass calculated as dry weight/unit area.

Results

Nail thickness

The terminal nail thickness was comparable in the two different nails examined in each of two of the patients but varied from 0·79 to 2·0 mm between the nails from different subjects. Regardless of absolute thickness, each of the 21 nails examined from 19 different patients showed the same site variation. Nail thickness was 0·66±0·09 mm at the mid-point of the germinal matrix, 1·24±0·09 mm at the proximal nail bed ($P<0{\cdot}002$) and 1·53±0·10 mm at the most distal nail site ($P<0{\cdot}004$) (Fig. 2). This corresponds to 43% of final nail thickness at the mid-point of the lunula (germinal matrix) and 81% of final nail thickness at the proximal nail-bed area, the final 19% of nail thickness being added along the nail bed beyond the germinal matrix.

Nail mass

Nail mass was similarly increased from 52±4 mg/cm^2 over the germinal matrix, to 70±5 mg/cm^2 over the proximal nail-bed area ($P=0{\cdot}0002$), with a further increase to 87±10 mg/cm^2 ($P=0{\cdot}0061$) from the proximal nail bed towards the distal free edge of the nail, corresponding to a mean increase in mass of 20% between the proximal and distal end of the nail beyond the germinal matrix.

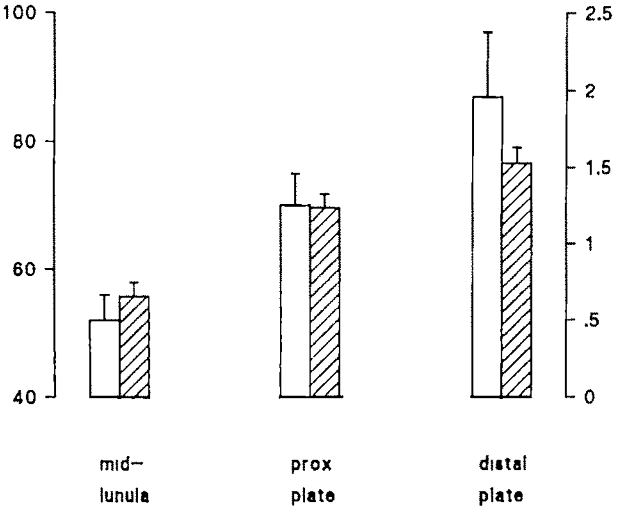

Figure 2. Nail thickness and mass at the three points and zones sampled showing progressive increase in both along the length of the nails. □, nail mass (mg/cm^2); ■, nail thickness (mm).

Discussion

We have shown that nail thickness and mass increased both from the central lunula to the distal margin of the lunula and also, to a lesser extent, to the distal free edge of the nail. The increase in thickness occurred in all the nails measured and was always much greater than the error of the measurement. Nail thickness increased rapidly over the lunula, doubling from 43 to 81% of its final thickness from the mid-point to the end of the lunula, the remaining 19% of terminal thickness being contributed along the nail bed. These findings demonstrate that a single measurement of nail thickness is inadequate for comparisons unless it is specifically related to the site of measurement along the nail.

To exclude the possibility that the increase in thickness was due to simple expansion, we measured dry weight/unit area and found that it also increased, 20% of nail mass being contributed by the nail bed beyond the lunula. Thus the increase in nail thickness was due to increased nail formation and not simply to expansion or hydration. In preliminary studies of three nails, protein content was found to increase in proportion to nail mass, again in keeping with increased nail synthesis. We therefore conclude that the increase in nail thickness and mass is due to continued nail formation. The measurements show this to be very rapid in the proximal nail over the lunula, indicating a very fast rate of production of cells in the germinal matrix in this region. The ventral nail, which is formed subsequently and more slowly by the nail bed, nevertheless contributes 20% of the final nail mass. This surprisingly large contribution of ventral nail is a normal occurrence because the nails studied were all healthy and had only been removed following an injury. Although we measured nail thickness at only three points the changes in nail mass encompassed the intermediate points, showing that growth is continuous along the whole of the nail bed.

Previous evidence on the contribution of the nail bed to nail production is conflicting and contradictory as are the textbook accounts.[10–13, 15] Earlier anatomical and pathological studies[1,3–5] provided evidence of a ventral component from the nail bed and a dorsal component from the underside of the nail fold in addition to the bulk of the nail originating in the matrix. This evidence was challenged by Zaias and Alvarez[8] and Norton[9], who found no evidence of a ventral nail contribution using autoradiographic distribution of radiolabelled thymidine and glycine in monkeys and man. Unfortunately both these studies were flawed. In particular the study of Zaias and Alvarez was inadequate with regard to site of delivery of the isotope and definition of the time and site of examination required to distinguish nail growing out from the matrix from that produced by the nail bed. Likewise, although Norton injected the isotope subungually into the matrix, the description of the sequence of labelling along the nail bed was incomplete and the clear findings of incorporation, particularly of glycine, by the cells of the nail bed were inexplicably dismissed. For these reasons it cannot be excluded that the isotopic incorporation found in the nail bed in both studies was due to formation of ventral nail by the nail bed. These studies cannot therefore be regarded as definitive, although they have been widely quoted. Likewise recent ultrasound studies[13] have been interpreted incorrectly as showing no evidence of a ventral nail when they simply show no evidence of a corresponding sound reflection.

Thus, our finding that 20% of the nail mass is formed by the nail bed as the nail grows out along it resolves the previous conflict and is the first definitive quantitative demonstration of the contribution of the ventral nail to the nail plate. We believe this to have considerable implications for nail disease and for the penetration of drugs into diseased nail.[16]

References

1 Lewis BL. Microscopic studies of foetal and mature nail and surrounding soft tissue. *Arch Dermatol Syphilol* 1954; **70**: 732–44.

2 Samman PD. The ventral nail. *Arch Dermatol* 1961; **84**: 1030–3.

3 Achten G. L'ongle normal et pathologique. *Dermatologica* 1963; **126**: 229–45.

4 Hashimoto K, Gross BG, Nelson R, Lever WF. The ultrastructure of the skin of human embryos. III. The formation of the nail in 16–18 week old embryos. *J Invest Dermatol* 1966; **47**: 205–17.

5 Jarrett A, Spearman RJC. The histochemistry of the human nail. *Arch Dermatol* 1966; **94**: 652–7.

6 Zaias N. The movement of the nail bed. *J Invest Dermatol* 1967; **48**: 402–3.

7 Caputo R, Dadati E. Preliminary observations about the ultrastructure of the human nail plate treated with thyioglycolic acid. *Arch Klini Exp Dermatol* 1968; **231**: 344–54.

8 Zaias N, Alvarez J. The formation of the primate nail plate. An autoradiographic study in squirrel monkey. *J Invest Dermatol* 1968; **51**: 120–37.

9 Norton LA. Incorporation of thymidine-methyl-H^3 and glycine-2-H^3 in the nail matrix and bed of humans. *J Invest Dermatol* 1971; **56**: 61–8.

10 Achten G. Histopathology of the nail. In: *The Nail* (Pierre M, ed), 2nd edn. G.E.M. Monograph 5, Edinburgh: Churchill Livingstone, 1982; 1–4.

11 Runne U, Orfanos CE. The human nail: structure, growth and pathological changes. *Curr Probl Dermatol* 1981; **9**: 102–49.

12 Dawber RPR, Baran R. The nails. In: *Textbook of Dermatology* (Rook A *et al.*, ed), 4th edn. Oxford: Blackwell Scientific Publications. 1986; 2039–43.

13 Jemec GBE, Serup J. Ultrasound structure of the human nail plate. *Arch Dermatol* 1989; **125**: 643–6.

14 Samman PD. In: *The Nails in Disease.* William Heinemann Medical Books Ltd, 1980; 2–14.

15 Zaias N, Baden HP. Nails. In: *Dermatology in General Medicine.* (Fitzpatrick T, Eisen AA, Wolff K, Freedberg IM & Austen FK, eds) New York: McGraw Hill Book Co., 1979; 418–37.

16 Munro CS, Rees JL, Shuster S. Short duration terbinafine therapy penetrates diseased distal nail via the ventral nail bed and is effective in onychomycosis. *Br J Dermatol* 1990; **123**: 825.

British Journal of Dermatology (1991) 125, 30–37. ADONIS 000709639100160L

Measurement of cutaneous inflammatory reactions using a scanning laser-Doppler velocimeter

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Accepted for publication 15 January 1991

Summary

The performance of a new scanning laser-Doppler velocimeter (LDV), which can rapidly measure blood flux over a large area of skin without contact with the skin surface, was compared with that of a conventional laser-Doppler instrument. The vascular response was measured to a range of doses of UVB, and dilutions of contact allergens and sodium lauryl sulphate. The detection threshold of the scanning LDV was equal to, or lower than, that of the conventional instrument. For allergic contact hypersensitivity reactions (ACH), the coefficient of variation was significantly less using the scanning LDV.

The scanning LDV allowed accurate measurement of the change in area that occurs with increasing intensity of inflammatory reaction. For ACH reactions the area of inflammation continued to increase at dilutions where blood flux had reached a plateau. The flare area was found to increase linearly with log dose of histamine with no change in blood flux.

Laser-Doppler velocimetry (LDV) has been used widely over the last 10 years as a non-invasive method for measurement of the vascular changes accompanying cutaneous inflammation[1–3] and a number of LDV instruments are now available commercially. The principle of LDV is based upon measurement of the frequency change of monochromatic red light that has been scattered by moving red blood cells. In conventional instruments the laser light is transmitted to and from the skin surface by a fibre-optic light guide, positioned over the area of interest and secured with adhesive tape. Although these instruments readily permit continuous and repeated measurement of blood flux at a single point on the body surface, they are limited by the small area that can be measured at one time. Study of inflammation at multiple sites on the skin is therefore time-consuming as the light guide has to be repositioned between each measurement.

A laser-Doppler instrument has been developed which can measure cutaneous blood flux over a large area of skin, within a short time period, and without contact with the skin surface. We have evaluated this new scanning LDV by comparing its performance in the measurement of four different cutaneous inflammatory reactions with that of a conventional laser-Doppler instrument.

Methods

Inflammatory responses

The following inflammatory reactions were studied:

UVB erythema. The erythemal response to ultraviolet (UV) B radiation was measured in four adult volunteers (sun-reactive skin type II or III). Eight or nine adjacent sites of 10-mm diameter on the back were irradiated with a geometric series of doses of UVB (the maximum dose used being approximately four times the minimal erythema dose) using a filtered medium pressure mercury arc lamp.[4] Responses were measured at 24 h.

Allergic contact hypersensitivity (ACH). Four patients who were known to have positive reactions to at least one common antigen were challenged with a series of dilutions of the appropriate antigen as described previously.[5] Two patients were sensitive to nickel, one to wool alcohols and one to thiuram mix. Nickel sulphate was prepared as a 20% solution in water; solutions of wool alcohol and thiuram mix were prepared by dissolv-

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This work was presented in part at the 1990 meeting of the British Society for Investigative Dermatology.

ing 200 mg of allergen (as supplied for routine patch testing by Trolab, Hermal, Hamburg, Germany) in 0·8 ml of chloroform. Successive dilutions (range 1 in 5 to 1 in 5×10^6 of the concentration of allergen used in our standard patch-test series) were prepared in water or chloroform as appropriate and 20 μl of each dilution pipetted on to an Al-test (Imeco) and allowed to evaporate. Patches were applied to the volar aspect of the forearm and secured with adhesive tape. The patches were removed after 48 h and the responses measured at 72 h.

Irritant reaction. Six successive twofold dilutions of a 4% solution of sodium lauryl sulphate (SLS) were prepared; 15 μl of each dilution were pipetted on to a filter paper disc and Finn chamber, and applied to the volar aspect of the forearm of four adult volunteers. The patches were removed after 48 h and the responses measured at 72 h.

Histamine induced weal and flare. Histamine acid phosphate (0·5, 2, and 8 μg in 100 μl of saline) was injected intradermally into the volar aspect of either forearm in four adult volunteers.

Scanning LDV measurements

The principle of measurement of blood flux with the scanning LDV is no different from that of a conventional LDV instrument. However, with the scanning LDV, light from a helium–neon laser is reflected by a mirror on to an area of skin and the light remitted from the skin is returned by the mirror and a series of lenses to four photodiodes. The mirror is mounted in a motor-driven gimbal which permits the light to scan the area of interest in a rectilinear manner (Fig. 1). The signal from the photodiodes is first processed to form a scale with 256 subdivisions. This is then displayed on a monitor screen as an image of 250 × 250 pixels where blood flux is shown on a colour scale with 16 subdivisions. The distance between the mirror and the skin surface is not critical, allowing whole body or more detailed small-area scans to be performed. At the closest scanning distance of 0·6 m, a skin area of 0·023 m^2 is defined, each pixel then representing a skin surface area of approximately 1 mm^2. Each scan is completed in 6 min irrespective of area under study. A full technical description of the instrument has been published.[6]

Scans of UVB, ACH and irritant inflammatory reactions were performed in triplicate. Histamine-induced reactions were only scanned once in view of the more rapid change in reaction with time. A square of matt black adhesive tape (400 mm^2) positioned within the scan area enabled the pixel area for each scan to be calculated.

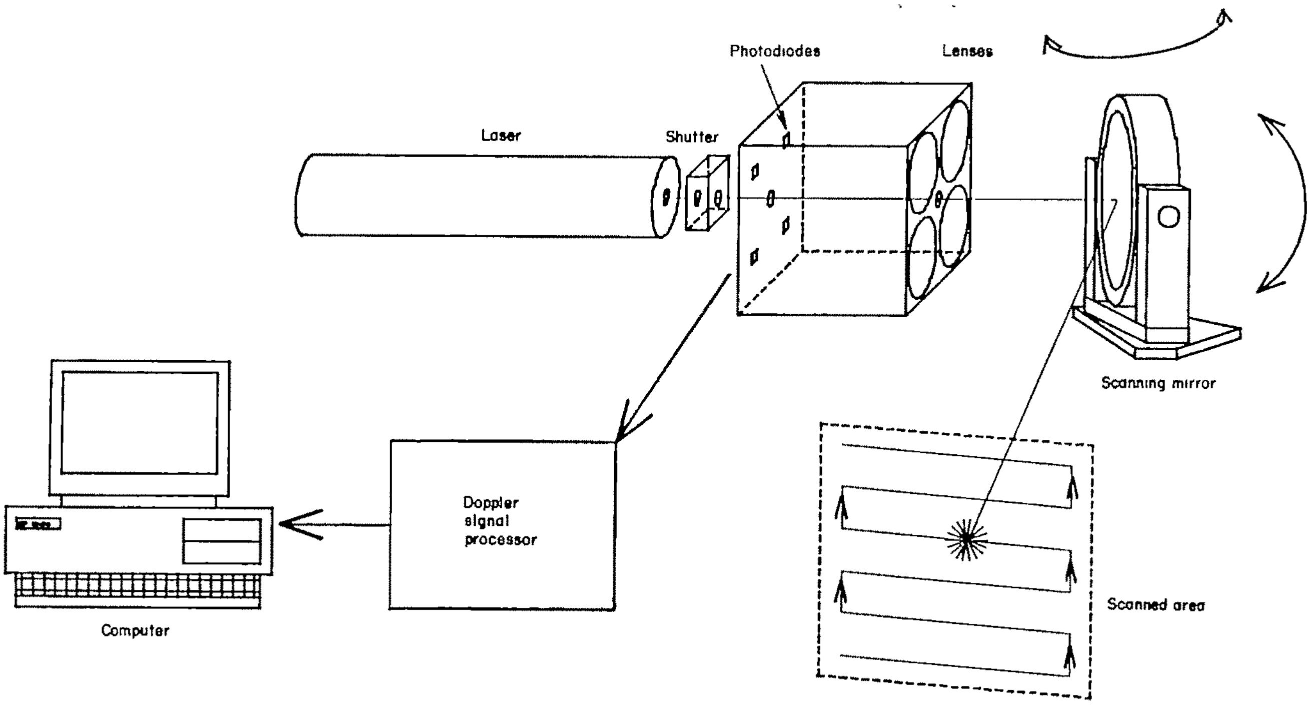

Figure 1. Block diagram of the scanning laser-Doppler velocimeter.

Conventional LDV

Blood flux was measured using a commercially available laser-Doppler velocimeter (Periflux PF2, Perimed, Sweden). Measurements of the different inflammatory reactions were made at each site in triplicate, the probe holder and probe being repositioned between each measurement. Randomly selected, adjacent, untreated sites were used as controls. The reading from the instrument was relayed to a chart recorder and the mean deflection estimated visually from the recording produced at each site over a period of at least 20 s after steady-state conditions had been achieved. The increase in flux due to inflammation (ΔF) was expressed as the difference between the mean reading at each site and the mean background reading determined at three adjacent control sites.[7]

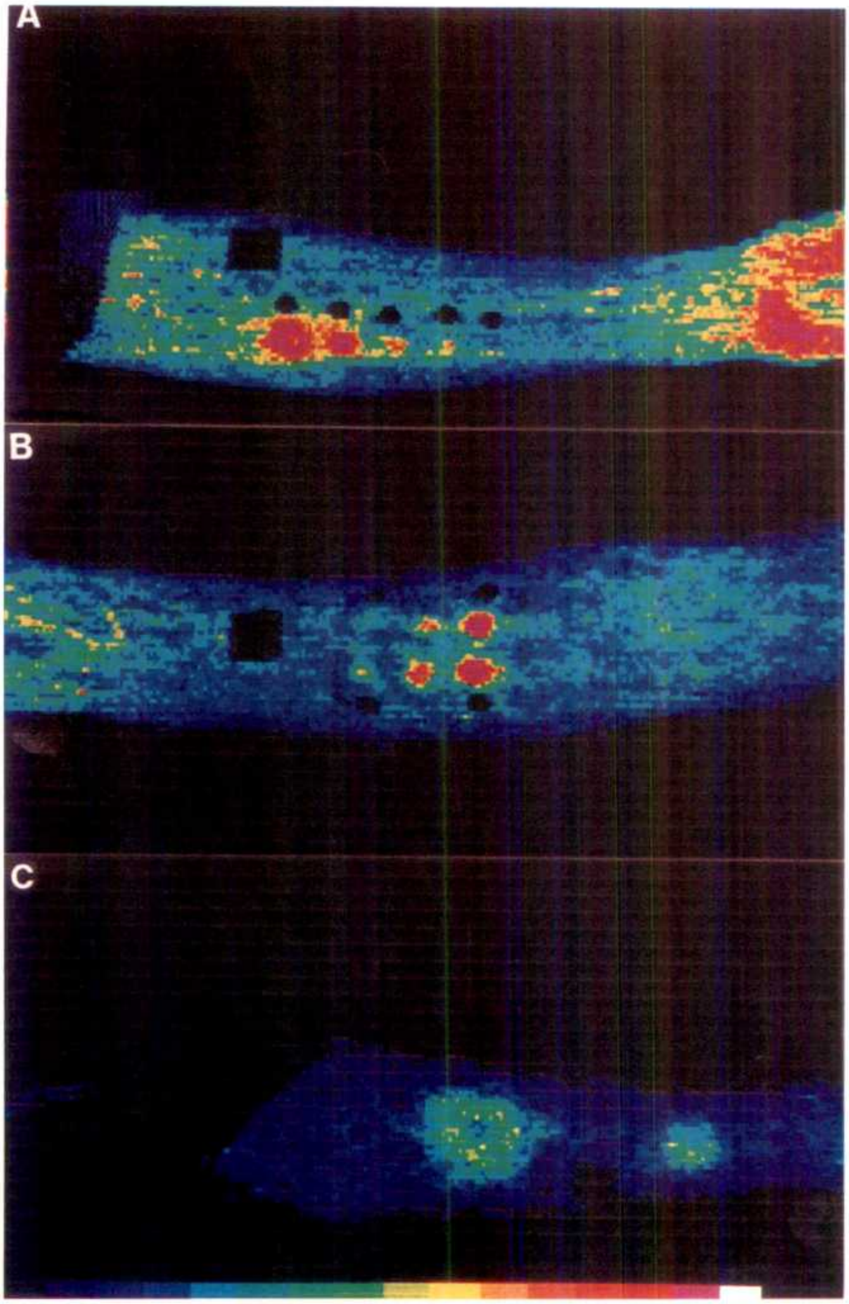

Figure 2. Representative scans of (a) ACH, (b) irritant and (c) histamine induced reaction.

Results

Representative scans of the inflammatory reactions are shown in Figure 2.

Quantification of scanning LDV-generated data

The reference area of 400 mm^2 defined with matt black adhesive tape squares allowed calibration of pixel area for each scan. A rectangle at least four times the area of the tape was outlined on an adjacent area of the scan that corresponded to normal skin. The mean flux within this area was defined as 'the mean background flux'. The 10% of pixels representing the highest blood flux within the background area were identified, and the mean blood flux of this group was defined as 'maximum background flux'.

A similar technique was used to calculate a 'maximum flux' value for each inflammatory skin reaction. The increase in flux at each site due to inflammation (ΔF) was expressed as the difference between 'maximum flux' and 'maximum background flux'.

The area of the inflammatory reaction at each site was calculated from the number of pixels showing a blood flux value greater than two standard deviations above the mean background flux.

UVB, allergic contact hypersensitivity and irritant reactions

Dose–responses

The ΔF values obtained for each set of readings from both instruments were plotted against log dose of UVB, antigen or irritant. A sigmoid dose–response relationship was found in all cases (Fig. 3). The scanning LDV also allowed dose–response curves to be plotted using area of inflammation as a measure of response. UVB and

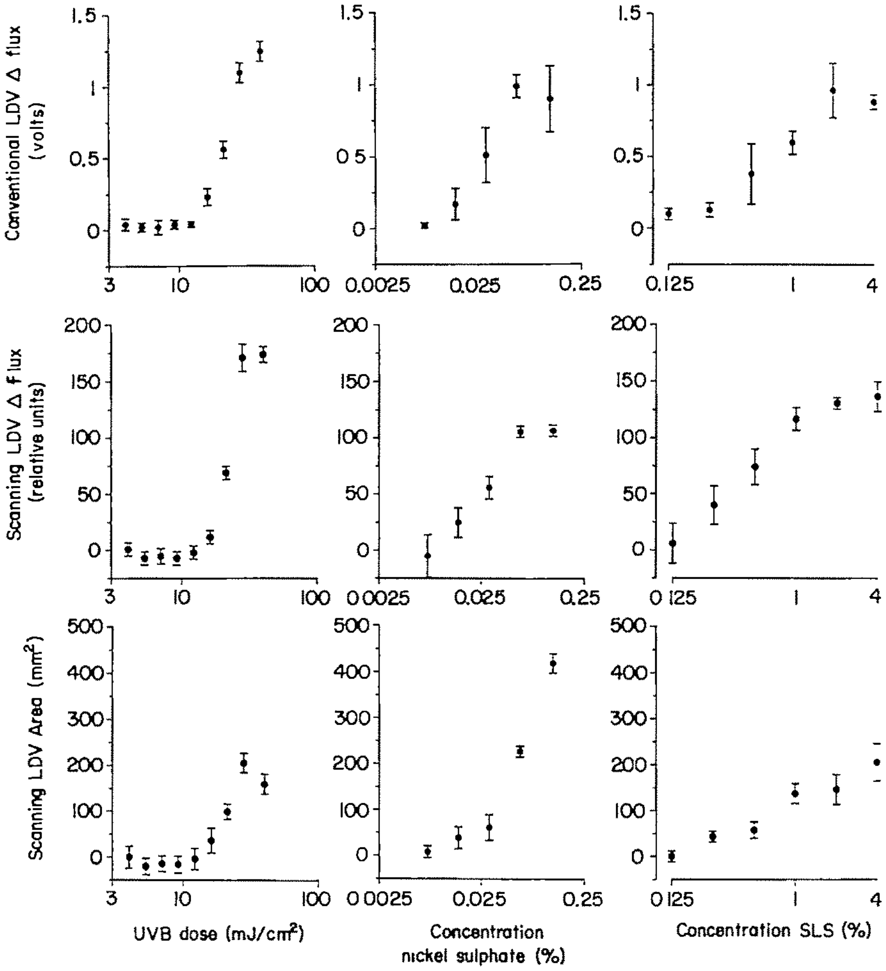

Figure 3. Examples of dose–response curves for UVB, ACH and irritant inflammatory reactions. Each point represents the mean ± SD of the increase in blood flux or the area of inflammation.

Table 1. Smallest dose or dilution producing minimal erythema or significant change in blood flux with either instrument

	Measurement method		
	Conventional LDV	Scanning LDV	Visual threshold
UVB (mJ/cm^2)			
1	15	11·3	11·3
2	16·1	16·1	12·2
3	12·2	12·2	9·2
4	12·2	12·2	9·2
ACH (dilution)			
1	1/5000	1/5000	1/5000
2	1/500	1/5000	1/500
3	15/1000	15/1000	7·5/1000
4	1/500	1/50,000	1/5000
Irritant (% concentration of SLS)			
1	1·0	0·5	0·25
2	0·25	0·125	0·125
3	0·125	0·25	0·125
4	1	1	1

irritant-induced reactions all showed sigmoid responses. However, for three of the four ACH reactions the area of inflammation continued to increase at dilutions where the increase in blood flux had reached a plateau.

Correlation between the conventional LDV and the scanning LDV

The values of ΔF obtained with the scanning LDV showed a significant correlation with those obtained with the conventional LDV for UVB, ACH and irritant reactions (Spearman-rank correlation coefficient, $P<0{\cdot}001$).

Comparison of threshold sensitivity and coefficient of variation

The smallest dose of UVB or SLS, or largest dilution of antigen, to produce a significant increase in blood flux over background levels (defined as $P<0{\cdot}05$, *t*-test) with either instrument was used to compare their sensitivities. The detection threshold of the scanning LDV was equal to or lower than that of the conventional LDV for all the UVB and ACH reactions, and three of the four irritant inflammatory reactions (Table 1).

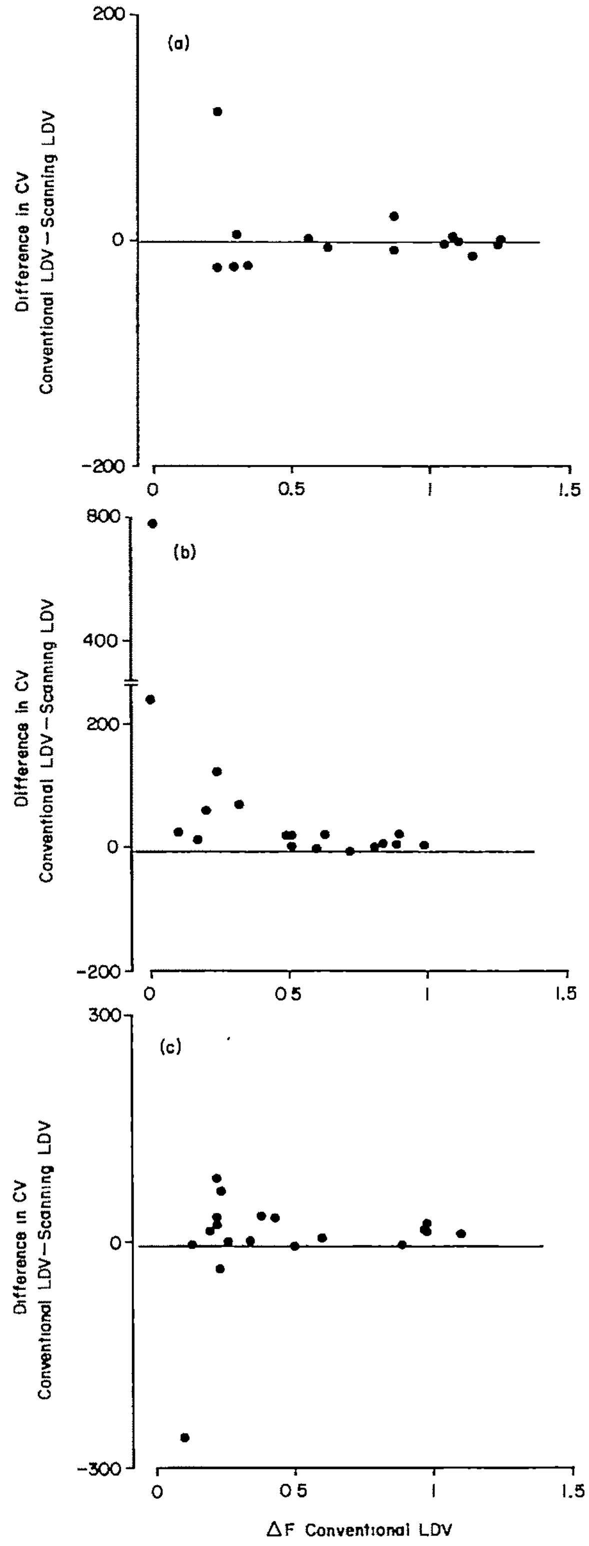

Figure 4. The relationship between coefficient of variation (CV), expressed as CV conventional LDV minus CV scanning LDV, and the intensity of the inflammatory reaction. (a) UVB, (b) ACH and (c) irritant reactions.

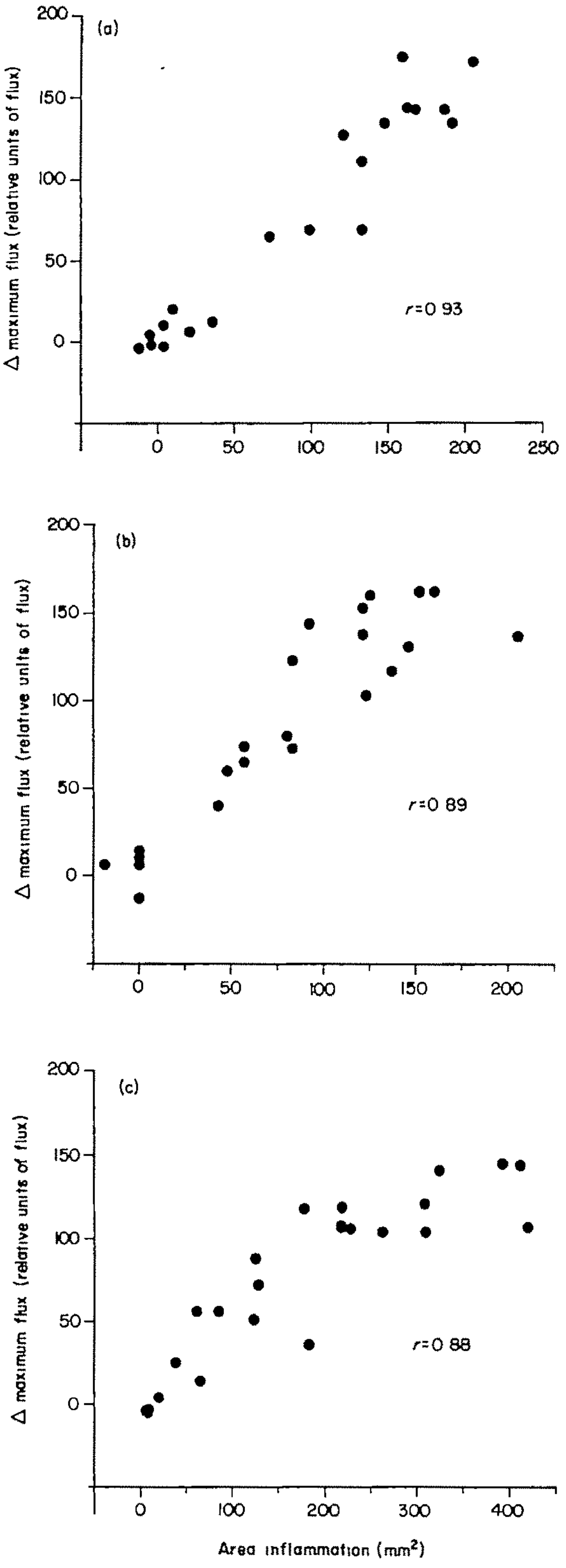

Figure 5. Correlation between ΔF and area of inflammatory reaction measured with scanning LDV for (a) UVB, (b) ACH and (c) irritant reactions.

The coefficient of variation (CV) was calculated for both instruments at each skin site. The difference between the CV obtained with the scanning LDV and the conventional LDV was plotted against intensity of reaction (ΔF for conventional LDV, Fig. 4) and the sign test used for statistical analysis. There was no significant difference in the CV between the conventional and scanning LDV for UVB and irritant inflammatory reactions. However, for ACH reactions the CV was significantly less for the scanning LDV ($P<0{\cdot}001$) than for the conventional LDV. This difference was most evident for inflammatory reactions at or just above threshold.

Correlation between ΔF and area of inflammatory reaction measured with SLD

The relationship between ΔF and area of inflammation for UVB, ACH and irritant reactions is shown in Figure 5. ΔF values showed significant correlation with the area of inflammation values for UVB, ACH and irritant-induced inflammation (Spearman-rank correlation coefficient, $P<0{\cdot}001$). ACH reactions differed from UVB and irritant ones in that the relationship appeared to be non-linear in the more intense reactions with a plateau observed where the area of inflammation continued to increase with no increase in ΔF.

Measurement of response to intradermal histamine

The flare response but not the weal was clearly defined using the scanning LDV (Fig. 2). Flare area, calculated as described above, increased linearly with log dose of histamine (Fig. 6). The maximum blood flux within the area of flare, however, showed no increase with increasing dose of histamine.

Discussion

The performance of a new scanning laser-Doppler velocimeter has been compared with that of a conventional LDV instrument in the measurement of experimentally induced cutaneous inflammation. We chose to study UVB, ACH, chemical irritant and histamine-induced inflammation as these reactions differ greatly in clinical appearance, and therefore require different measurement techniques.

The scanning LDV was quick and simple to use, and produced hard copy, high-resolution colour scans. It was possible to analyse the data obtained from the instrument to give a measurement of maximum blood flux (equivalent to the reading obtained from conventional

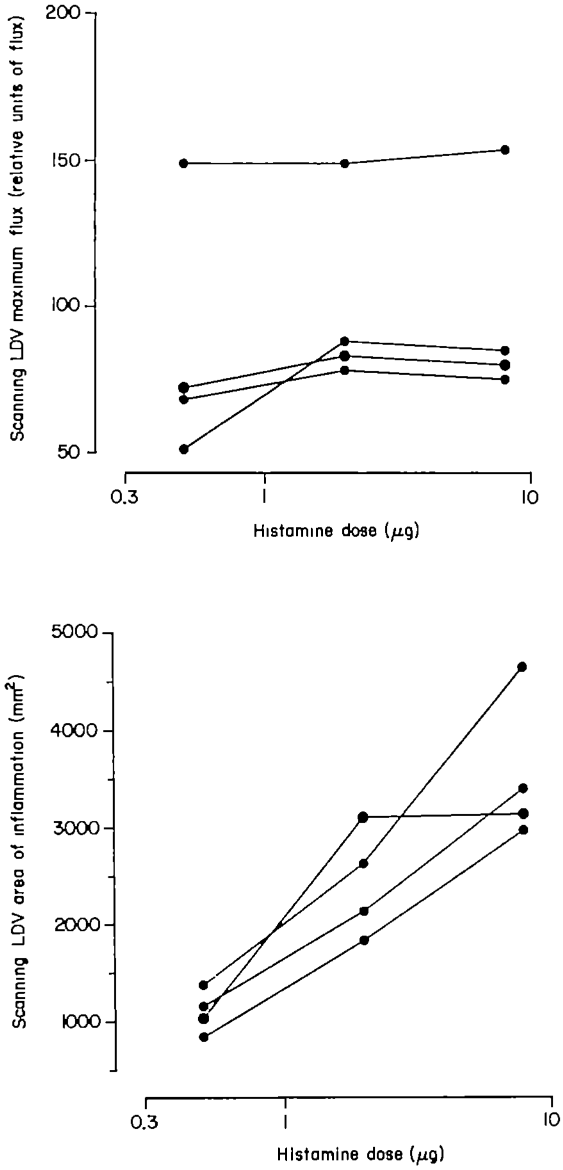

Figure 6. Relationship between ΔF and area of inflammatory reaction and increasing histamine dose.

instruments) and also a measurement of the area of inflammatory reaction.

Using the maximum blood flux measurement, dose–response curves were constructed for UVB, ACH and irritant inflammation. The form of the dose–response curves was similar for both instruments (Fig. 3). Correlation analysis showed a significant relationship between the scanning and conventional instruments (Spearman rank correlation coefficient $P<0{\cdot}001$). Visual assessment of the data suggested that the relationship was non-linear for ACH and irritant inflammatory reactions. Maximum blood flux values obtained with the scanning LDV showed a larger increase initially before reaching a plateau, whereas blood flux values obtained with the conventional instrument continued to increase throughout the dose/dilution range used. It remains to be determined which of the two LD velocimeters gives the best representation of blood flux in these inflammatory reactions.

In terms of detection threshold the scanning LDV was equal to or more sensitive than the conventional instrument (Table 1). Triplicate measurements allowed us to calculate a coefficient of variation for the measurement of the inflammatory response at each skin site. There was no significant difference between CV values for the two instruments when measuring UVB or irritant responses. However, for ACH measurements the scanning LDV showed significantly lower CV values than the conventional LDV (Fig. 4), and this difference was most evident at reactions at or just above the detection threshold. As the conventional LDV only measures blood flux within an area of approximately 2 mm diameter, small changes in site of measurement when repositioning the probe may be expected to result in inaccuracy, especially when measuring a non-uniform skin reaction such as ACH.

The area of inflammation increases with the intensity of the reaction. This may be seen in the clinical evaluation of patch-test responses where strong positive reactions extend to involve an area much larger than that to which the allergen had been applied. The area of reaction can be measured approximately using conventional LDV instruments only if repeated closely apposed readings of blood flux are made; see for example Swanson–Beck and Spence 1986.[8] The scanning LDV allows for the first time a quick and simple method of measuring the area of inflammation. Dose–response curves showed that the area of inflammation increases linearly with log dose for UVB, ACH and irritant reactions (Fig. 6). In three of the four ACH reactions, the area of inflammation continued to rise at doses above which blood flux had reached a plateau, suggesting that area measurements may provide more information concerning the full dose–response relationships for ACH than those based on maximum blood flux measurements.

The scanning LDV clearly defined the flare but not the weal component of the response to injected histamine. As the edge of the histamine-induced flare is indistinct and irregular, the conventional method of calculating flare area by measurement of diameter is difficult and subjective.[9] The scanning LDV allowed objective measurement of flare area which was shown to increase linearly with log dose of histamine (Fig. 6). Despite the increase in flare area, the maximum blood flux within the flare showed no significant change with dose of histamine.

The new scanning LDV instrument represents an important advance in the measurement of blood flux using laser-Doppler techniques. It is as sensitive as conventional instruments but has the considerable advantage of being able to measure blood flux over large areas without contact with the skin surface. The measurement of area of inflammation using the instrument will have many dermatological applications, including the assessment of the severity and the response to treatment of skin diseases characterized by an increase in blood flow, such as psoriasis.

Acknowledgment

We thank Merrel Dow Pharmaceuticals Ltd for financial support towards the cost of reproducing Figure 2 in colour.

References

1 Holloway GA, Watkins D. Laser Doppler measurement of cutaneous blood flow. *J Invest Dermatol* 1977; **69**: 306–9.

2 Nilsson GE, Otto U, Wahlberg JE. Assessment of skin irritancy in man by laser-Doppler flowmetry. *Contact Dermatitis* 1982; **8**: 401–6.

3 Staberg B, Klemp P, Serup J. Patch test responses evaluated by cutaneous blood flow measurements. *Arch Dermatol* 1984; **120**: 741–3.

4 Farr PM, Diffey BL, Humphreys F. A quantitative study of the effect of terfenadine on cutaneous erythema induced by UVB and UVC radiation. *J Invest Dermatol* 1986; **87**: 771–4.

5 McLelland J, Shuster S. Contact dermatitis with negative patch tests. *Br J Dermatol* 1990; **122**: 623–30.

6 Essex TJH, Byrne PO. A laser Doppler scanning technique for imaging blood flow in the skin. *Measurement Science and Technology* 1991; (in press).

7 Farr PM, Diffey BL. The vascular response of human skin to ultraviolet radiation. *Photochem Photobiol* 1986; **44**: 501–7.

8 Swanson Beck J, Spence VA. Patterns of blood flow in the microcirculation of the skin during the course of the tuberculin reaction in normal human subjects. *Immunology* 1986; **58**: 209–15.

9 Forrest MJ, Williams TJ. Blood flow—including microcirculation. In: *Pharmacology of the Skin* (Greaves MW, Shuster S, eds), 1st edn, Vol. I. Berlin: Springer Verlag, 1989; 117.

British Journal of Dermatology (1991) **125**, 38–42. ADONIS 000709639100161Y

Altered cell-mediated immunity to group A haemolytic streptococcal antigens in chronic plaque psoriasis

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Accepted for publication 18 January 1991

Summary The proliferative lymphocyte response to sonicated group A, β-haemolytic streptococci (Strep-A) was measured by thymidine incorporation in 78 patients with psoriasis (guttate, chronic plaque or both). Lymphocytes from 72 of these patients were also cultured with streptokinase/streptodornase (SK/SD), and 20 of the patients with chronic plaque psoriasis were further tested with PPD, *Candida albicans* and sonicated *Streptococcus mutans*, a bacterial type not associated clinically with psoriasis. The median stimulation index (SI) of the psoriasis group to the Strep-A preparation was significantly higher than that of a group of 27 non-psoriatic individuals ($P<0{\cdot}05$). Within this group, only the patients with chronic plaque psoriasis ($n=42$) showed a significantly increased proliferative response compared to the non-psoriatic controls (median SI$=123{\cdot}8$ and $31{\cdot}9$, respectively, $P<0{\cdot}01$). Although the lymphocyte response of the chronic plaque group to SK/SD was also markedly higher than that of the control group, this difference did not reach statistical significance. In addition, these patients did not show significantly increased responses to any of the other antigens tested, including *S. mutans*.

No correlation was observed between the degree of proliferation to Strep-A and disease extent or activity. Similarly, ASO titres, which were raised in 11 out of 23 guttate and three out of nine chronic plaque psoriasis patients tested, did not correlate with the proliferative responses observed.

The association between psoriasis and β-haemolytic streptococcal infections is well documented. Patients with guttate psoriasis show a strong association between previous streptococcal throat infections, raised antibody titres to streptococcal antigens and the initiation of skin lesions.[1] In addition, exacerbation of chronic plaque (CP) psoriasis may occur following tonsillitis.[2] Therefore several reports of elevated antistreptolysin titres[1,3] and/or positive group A, β-haemolytic throat cultures[3,4] in some patients with CP psoriasis suggest that haemolytic streptococci may act not only as a triggering factor for self-limiting acute psoriasis, but also as an on-going stimulus for the chronic form of the disease.

Based on this premise, Rosenberg *et al.*[5] treated nine patients with resistant, streptococcal-associated psoriasis with rifampicin combined with penicillin or erythromycin to determine the effect of streptococcal eradication on their disease. Of the nine patients, four of whom had CP psoriasis, five were evaluated as having an excellent response (95–100% disappearance of lesions) and four as having a good response (80–95% improvement), indicating that streptococcal antigens may be involved in persistent psoriasis.

Furthermore, patients with psoriasis appear to have an altered response to streptococcal antigens *in vivo* as demonstrated by a decrease in the intensity of erythema and induration 48 h after intradermal injection of streptokinase/streptodornase (SK/SD).[6] Patients with psoriasis also show a delay in resolution of this delayed hypersensitivity response compared with non-psoriatic controls.[7] *In-vitro* cell-mediated immunity to group A streptococcal cell wall and cell membrane antigens as measured by the two-step leucocyte migration inhibition assay was shown to occur significantly more often in patients with psoriasis than in controls, but this response was restricted to patients with guttate psoriasis.[8] Increased humoral responses to extracellular products of group A streptococci have also been reported in psoriasis[9] as in patients with other complications of group A streptococcal infections, such as rheumatic fever.[10]

The purpose of this study was to examine the proliferative response of blood lymphocytes from patients with different types of psoriasis to group A

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haemolytic streptococci and to compare this with responses to other antigens, including *Streptococcus mutans* that has not been implicated in psoriasis.

Methods

Patients and controls

Seventy-eight patients (44 males and 34 females) with psoriasis were studied. They were subdivided into three groups according to the following clinical criteria: (i) guttate psoriasis defined as a sudden outbreak of small lesions less than 5 mm in diameter on the trunk; (ii) chronic plaque (CP) psoriasis defined as the presence of stable lesions; (iii) guttate+CP psoriasis in which a guttate flare occurred in patients with a history of or existing CP lesions.

There were 24 guttate patients (age 16–64 years), 42 CP patients (age 15–85 years) and 12 guttate+CP patients (age 20–52 years). Of the total of 36 patients with guttate psoriasis, 15 were receiving treatment at the time of the study; one patient was taking dithranol, two were receiving photochemotherapy (PUVA) and 12 were on topical steroids. Four of the 42 CP patients were receiving topical steroids and one PUVA. Seven patients were studied both on and off treatment.

Control subjects were 27 healthy adult members of staff who had no known history of psoriasis (14 males and 13 females).

Isolation of peripheral blood mononuclear cells (PBMC)

Peripheral blood mononuclear cells (PBMC) were isolated from 20 ml of heparinized blood by Ficoll–Hypaque gradient centrifugation (Lymphoprep, Nyegaard and Co., A/S. Oslo, Norway). The cells were then washed twice with RPMI 1640 (Flow Labs, Irvine, Ayrshire, Scotland) containing 2 mg/ml bicarbonate, 20 mM glutamine, 0·1 mg/ml streptomycin and 100 U/ml crystapen, and resuspended at 10^6 cells/ml in the same medium supplemented with 5% heat-inactivated autologous plasma.

Antigens

PBMCs from 78 patients with psoriasis were tested with group A, β-haemolytic streptococci (Strep-A); 72 were also cultured with SK/SD, and a proportion of CP patients were tested with purified protein derivative (PPD) (18 patients), *Candida albicans* and *S. mutans* (17 patients).

Strep-A (a mixture of four throat group A isolates) and *S. mutans* (a gift of Professor Lehner, Department of Immunology, Guys Hospital Medical School, London, U.K.) were prepared in the same manner. The bacteria were grown in Todd–Hewitt broth at 37°C for 24 h, harvested by centrifugation, inactivated by heating at 56°C for 1 h and washed four times in phosphate-buffered saline. The bacterial cells were adjusted to approximately 1 mg protein/ml and then sonicated for a total of 10 min in 1-min bursts, aliquoted and stored at −20°C. The bacterial preparations were then added to PBMC cultures at dilutions of 1:100, 1:1000 and 1:10,000 in plasma-supplemented culture medium.

The PBMCs were cultured with SK/SD (Lederle, Gosport, Hants, U.K.) at final concentrations of 25, 50 and 100 U/ml, with PPD (Central Veterinary Laboratories, Weybridge, Surrey) at 0·1, 1 and 10 μg/ml and with *C. albicans* (Central Public Health Laboratories, Colindale, U.K.) at 1, 10 and 100 μg/ml.

Proliferation assay

Triplicates of 10^5 PBMCs were set up with 100 μl of each antigen in flat-bottomed 96-well plates (Sterilin, Hounslow, U.K.) and cultured for 7 days in a humidified incubator with 5% CO_2/95% air at 37°C. Six hours before harvesting, each well was pulsed with 4 μCi/ml of tritiated thymidine, specific activity 5 Ci/mmol. Cells were harvested with an automatic cell harvester (Skatron, Lier, Norway) on to glass fibre filters and the amount of incorporated radioactivity was measured in a β-scintillation counter. Results were expressed as the Stimulation Index (SI), i.e. the ratio of the counts per min (c.p.m.) of the culture plus antigen to the c.p.m. of the culture with medium alone. The radioactivity of unstimulated cultures was, in most instances, below 500 c.p.m.

Anti-streptolysin-O titres

Thirty-two of the patients were tested on the same occasion for antistreptolysin-O (ASO) antibodies using a standard haemolysis inhibition assay. A titre of >200 IU/ml was taken as the upper limit of normal.

Statistical analysis

The differences between groups of data were analysed by the Wilcoxon two-sample test.

Results

Responses to Strep-A and SK/SD

A wide range of responses was observed when lymphocytes from the 78 patients with psoriasis and 27 non-psoriatic controls were stimulated with the Strep-A preparation (optimal dilution of 1:1000 in both instances), but a significantly stronger reactivity was demonstrated by the patients' lymphocytes overall ($P<0{\cdot}05$) (Fig. 1). This was mainly due to higher responses by lymphocytes from patients with CP psoriasis ($n=42$) who gave a median stimulation index (SI) of 124 compared with 32 for the non-psoriatic controls ($P<0{\cdot}01$). The responses of the patients with guttate psoriasis were not significantly different from those of either the non-psoriatic controls or the patients with CP psoriasis. The median SI for the 12 patients who had guttate + CP psoriasis was 74, whilst those with guttate psoriasis alone ($n=24$) had a median SI of 44 (Fig. 1). No difference in responses to Strep-A was observed in relation to treatment, and sequential studies of seven patients while on and off treatment showed fluctuations that were similar to those observed in untreated patients.

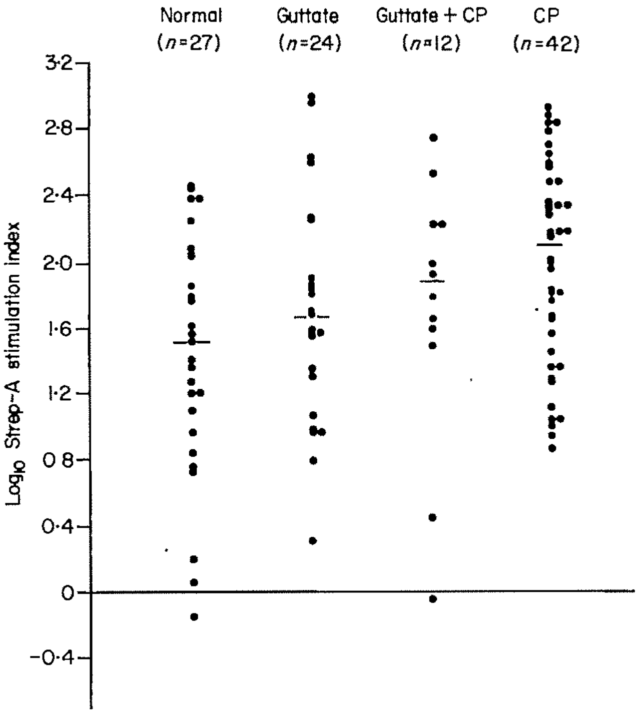

Figure 1. Proliferative responses expressed as $\log_{10}$ stimulation index to sonicated group A, β haemolytic streptococci (Strep-A), diluted 1:1000, of blood lymphocytes from patients with guttate, chronic plaque (CP) or guttate plus chronic plaque psoriasis compared to non-psoriatic controls. Median values are shown for each group.

When lymphocytes from patients with psoriasis were cultured with SK/SD antigen, the CP group had the highest median SI of 171, the guttate group the lowest median SI value of 51 and the patients with guttate + CP psoriasis an intermediary median SI value of 89. This response pattern was similar to that observed with Strep-A but the difference between the patient groups and the non-psoriatic controls (median SI = 61) was not significant for the SK/SD antigen. There was a positive correlation between the Strep-A and SK/SD responses for each group of patients ($r=0{\cdot}85$, $0{\cdot}65$, $0{\cdot}68$ for the CP, guttate, and CP + guttate groups, respectively).

To determine whether there was any relationship between lymphocyte responses and clinical changes, lymphocytes from 19 patients were tested with Strep-A and, in most instances, also SK/SD on two or three occasions over periods ranging from 2 weeks to 5 months. Nine non-psoriatic controls were similarly tested. The responses showed considerable variation, but this was similar in the psoriatic patients and the controls. No consistent relationship was observed between the clinical state and the proliferative lymphocyte responses in the patients.

Responses to S. mutans

As the patients with CP psoriasis showed the strongest responses to Strep-A, lymphocytes from 17 of these patients were cultured with a sonicate of *S. mutans*, a streptococcal organism that has not been associated with psoriasis. The median SI response to this preparation, at an optimal dilution of 1:1000, was higher for the CP patients than the 20 non-psoriatic controls (31 vs. 17) but this difference was not significant (Fig. 2). Thus, unlike the controls who responded similarly to both streptococcal preparations, the CP patients demonstrated significantly stronger stimulation by Strep-A than by *S. mutans* ($P<0{\cdot}005$).

Responses to non-streptococcal antigens

A wide range of proliferative responses to PPD and *C. albicans* was observed in both CP and non-psoriatic individuals. The median SI values for the CP group were higher (PPD: 190, $n=18$ vs. 144, $n=20$; *C. albicans*: 137, $n=17$ vs. 89, $n=20$ for CP and non-psoriatics, respectively) but these differences were not significant. Furthermore, the controls showed significantly stronger responses to PPD and *C. albicans* than to the Strep-A

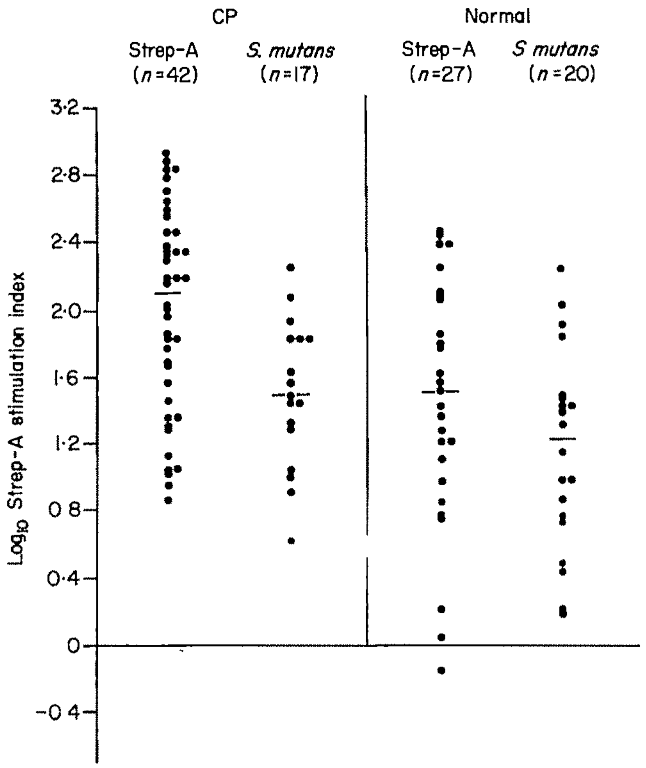

Figure 2. Proliferative responses expressed as $\log_{10}$ stimulation index to Strep-A and *S. mutans*, both diluted 1:1000, of blood lymphocytes from patients with chronic plaque psoriasis compared to non-psoriatic controls. Median values are shown for each group.

preparation ($P<0{\cdot}01$ and $P<0{\cdot}05$, respectively), whilst such a difference was not noted in the CP patients.

Anti-streptolysin-O titres

ASO titres were determined in 23 guttate and nine CP psoriasis patients at the time when their lymphocyte responses were measured. Eleven patients with guttate (49%) and three with CP (33%) psoriasis had raised ASO titres (>200 IU/ml). There was no correlation between the level of the ASO titre and the proliferative response to Strep-A in individual patients.

Discussion

This study has shown that blood lymphocytes from patients with CP but not guttate psoriasis have an enhanced proliferative response to sonicated group A, β-haemolytic streptococci compared with healthy, non-psoriatic individuals. This enhancement is selective for Strep-A as the responses to *S. mutans*, PPD and *C. albicans* were not significantly different between the groups. An earlier study, using a leucocyte migration inhibition assay, reported an increased lymphocyte response to streptococcal cell wall and cell membrane antigens, in patients with guttate but not other forms of psoriasis.[8] The reason for the discrepancy is not clear, but is probably due to different assay systems. The presence in the healthy control group of a small number of individuals with increased proliferative response to Strep-A is, perhaps, not surprising. Psoriasis is a common disorder and a high prevalence of psoriatic genes would be expected in the general population.

Our findings raise the question as to why patients with guttate psoriasis, in whom lesions commonly erupt 1–2 weeks after a streptococcal sore throat, do not show increased lymphocyte responses to Strep-A whilst patients with CP psoriasis do. This might be due to an active recruitment of Strep-A-reactive lymphocytes from the blood into the skin during the active phase of guttate psoriasis (the intermediary proliferative response of patients with both guttate and CP psoriasis to Strep-A is consistent with this possibility), an increase in circulating Strep-A-specific suppressor cells, or both.

Another possibility is that streptococcal antigens persist in CP psoriasis, perhaps due to a defect in clearance by the immune system. It is interesting that treatment of resistant, streptococcal-associated psoriasis with rifampicin combined with penicillin or erythromycin induced marked improvement of psoriatic lesions in nine patients, four of whom had CP psoriasis.[5] Monoclonal antibodies to group A streptococci have been shown to cross-react with several antigenic determinants in both normal and psoriatic skin[11] (I.Jonsdottir *et al.*, personal communication). Furthermore, the sequenced streptococcal M6 protein has a higher degree of homology with 50 kDa keratin type I than with other sequenced mammalian proteins, as shown by computer analysis (J McFadden *et al.*, personal communication). Both proteins have repeated segments of seven amino-acid sequences with only one amino acid difference, an arrangement that might be expected to give rise to cross-reactivity at the T-cell level because these cells recognize segments of primary (peptide) structures. The type I 50-kDa keratin is present in increased quantities and preferentially expressed in psoriatic skin.[12] Thus persistent activation of Strep-A-specific T lymphocytes in the throat could conceivably give rise to cross-reactive, autoimmune responses in the skin that might be involved in the maintenance of chronic psoriatic lesions. However, preliminary attempts to stimulate lymphocytes from patients with psoriasis with either a mixture of keratins or sonicated normal or psoriatic epidermal cell suspensions have been, for the most part, unsuccessful (data not shown).

The concept that molecular mimicry is responsible for the autoimmune manifestations that are associated with streptococcal infections may need to be modified with regard to the possibility that streptococcal M proteins are superantigens that activate subpopulations of human T cells.[13] It should be noted however that in this study, it is unlikely that the Strep-A preparation was acting as a superantigen because optimal proliferative responses were not observed until 5–7 days after initiation of the cultures[13] (data not shown).

It is possible that patients with CP psoriasis have increased lymphocyte reactivity to Strep-A because they are frequently or persistently colonized with group A streptococci to an abnormal degree without any causal relationship with their skin disease. However, we believe that the enhanced Strep-A lymphocyte reactivity in chronic psoriasis is more likely to be due to cross-reaction between streptococcal epitopes and skin components, and that this reactivity is not effectively suppressed in these patients.

Acknowledgments

This study was financed by grants from the Wellcome Trust to Drs L.Fry and B.Baker. Professor H.Valdimarsson was in receipt of grants from the Research Foundation of the University of Iceland.

References

1 Nørholm-Pedersen A. Infections and psoriasis. *Acta Derm Venereol (Stockh)* 1952; 32: 159–67.
2 Norlind R. Significance of infections in origin of psoriasis. *Acta Rheumatol Scand* 1955; 1: 135–44.
3 Haro HS, Patiala R, Widholm O. Psoriasis and streptococcal infections. *Ann Med Intern Fenniae* 1954; 43: 216–25
4 Cohen-Tervaert WC, Esseveld H. A study of the incidence of haemolytic streptococci in the throat in patients with psoriasis vulgaris with reference to their role in the pathogenesis of this disease. *Dermatologica* 1970; 140: 282–90.
5 Rosenberg EW, Noah PW, Zanolli MD *et al.* Use of rifampin with penicillin and erythromycin in the treatment of psoriasis. *J Am Acad Dermatol* 1986; 14: 761–4.
6 Krueger GG, Hill HR, Jederberg WW. Inflammatory and immune cell function in psoriasis—a subtle disorder. I. *In vivo* and *in vitro* survey. *J Invest Dermatol* 1978; 71: 189–94.
7 McFadden JP, Powles AV, Baker BS *et al* Intradermal antigen tests in psoriasis. *Acta Derm Venereol (Stockh)* 1990; 70: 262–4.
8 Gross WL, Packhauser U, Hahn G *et al.* Lymphocyte activation by streptococcal antigens in psoriasis. *Br J Dermatol* 1977; 97: 529–36.
9 Whyte JH, Baugham RD. Acute guttate psoriasis and streptococcal infection. *Arch Dermatol* 1964; 89: 350–6.
10 Read SE, Fischetti VA, Utermohlen V *et al.* Cellular reactivity studies to streptococcal antigens. *J Clin Invest* 1974; 54: 439–50
11 Swerlick RA, Cunningham MW, Hall NK. Monoclonal antibodies cross-reactive with group A streptococci and normal and psoriatic human skin. *J Invest Dermatol* 1986; 87: 367–71.
12 Hunter I, Skerrow D. Relationship of the final α-keratin compositions in dyskeratoses to those along the normal keratinization pathway: aetiological and diagnostic significance. *Br J Dermatol* 1989; 120: 363–9.
13 Tomai M, Kotb M, Majumdari G, Beachey EH. Superantigenicity of streptococcal M protein. *J Exp Med* 1990; 172: 359–62.

British Journal of Dermatology (1991) 125, 43–47. ADONIS 000709639100162D

Changes in the elastic tissue of the non-sun-exposed skin of cigarette smokers

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Accepted for publication 12 December 1990

Summary Biopsies were taken from the upper and inner arm of 10 60-year-old male cigarette smokers and compared with 10 age-matched controls who were non-smokers. The mean relative area, number and thickness of the elastic fibres were significantly increased in the cigarette smokers compared to the controls. These results were confirmed using antibodies to elastin or the microfibrillar component of elastic tissue. In the smokers the broader and more fragmented elastic fibres in the skin were not as intensely stained as those of the non-smokers and the ultrastructural alterations of the elastic fibres were similar to those in solar elastosis.

Smoking of cigarettes is a known risk factor for the development of pulmonary and arterial disorders. The effects of cigarette smoking on the skin has received little attention, although it was suggested as early as 1856 by Solly, who described the pale sallow skin colour and more pronounced wrinkles on the faces of smokers.[1] Later, a highly significant statistical relationship was shown between wrinkling of the skin on the face and smoking.[2–6] The aim of this study was to evaluate abnormalities of the dermal elastic tissue of the non-sun-exposed skin of heavy cigarette smokers.

Methods

Patients

Punch biopsies 4 mm in diameter were obtained from the upper and inner part of the arms of 10 60-year-old male smokers. The average number of 'pack-years', of smoking one pack per day, was 42 ± 10 years. Similar biopsies were taken from 10 age-matched men. None of the controls had smoked or had diabetes, hypertension or any connective tissue disorder. All the patients denied having taken drugs that could affect their connective tissue such as corticosteroids, colchicine and D-penicillamine.

Morphometric analysis

All specimens were fixed in Bouin's solution and embedded in paraffin and serial 4-μm thick sections were cut using a microtome. The sections were stained with orcein and polyphenolic (+) catechin dye. The catechin-stained elastic fibres appeared as deep blue-black fibrils against a colourless background and were evaluated using morphometry.[7] A Leitz-texture analysis system (Leitz-TAS) was used for the semi-automatic analysis of the network of elastic fibres as previously described.[8] This analysis enabled us to evaluate the percentage area of the dermal tissue section occupied by the elastic fibres and the number of elastic fibres per mm^2 and to prepare a histogram of fibre length and diameter distributions. Because of the heterogeneous composition of the elastic fibre network, two computer programs were used. One was for the superficial papillary elastic tissue composed of vertical bundles of microfibrils extending vertically to the dermal–epidermal junction, and for the arciform elastic network connecting the vertical fibres with the deeper mature elastic network. With the second program the deeper elastic fibres of the dermis were analysed automatically on the whole field of the monitoring screen. For each program 20–25 high-power fields were examined.[9]

The thickness of the dermis was measured on sections stained with haematoxylin and eosin. A video camera transferred the microscopic field to the monitoring

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screen of a SAGA semi-automatic morphometric device (Biocom, Les Ulis, France) connected to a Compaq microcomputer. The biopsies were observed at a low magnification, ×2·5, and 5–10 determinations were made on each of them. These measurements were calibrated and the results obtained directly in microns.[10] All the results were statistically analysed using the Student's unpaired *t*-test.

Immunohistochemical techniques

Punch biopsies (4 mm) from the non-sun-exposed skin of the upper and inner arm of the smokers and from the controls were snap frozen in liquid nitrogen. A two-step standard immunofluorescence labelling technique was performed with the following antibodies: polyclonal rabbit anti-P amyloid component (Dakopatts, Paris)[11] diluted 1:30, mouse monoclonal HB8 (Immunotech, Marseille)[12] diluted 1:100, rabbit anti-human fibronectin (Institut Pasteur, Lyon)[13] diluted 1:20 and anti-human elastin antibodies (Table 1).[14] Using a radioimmunoassay technique, the cross-reactivity with human type VI collagen was found to be less than 0·04%. A fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (Silenus, Laboratoire Eurobio) or an FITC-coupled goat anti-mouse IgG (Southern Biotechnology Associates) were used as the secondary antisera.

Electron microscopy

Two skin samples from heavy smokers were processed for electron microscopy. The specimens were fixed in 2·5% glutaraldehyde in cacodylate buffer at pH 7·4 and then post-fixed in osmium tetroxide. They were later embedded in epoxy resin and ultrathin sections cut and stained with lead citrate and uranyl acetate and observed in a Philips EM 300 electron microscope.

Results

Morphometric analysis

A moderate elastosis was observed in the dermis of all the heavy smokers. The mature elastic fibres were more numerous and wide and more fragmented than those of the non-smokers. The superficial elastic network appeared to be similar in both groups (Fig. 1). These histological findings were confirmed by the morphometric analysis. In the cigarette smokers, the mean relative area of elastic fibres (19·11 ± 7%) and the total number of elastic fibres per mm^2 of dermis (4551 ± 716) were significantly increased compared to the mean relative area of elastic fibres (9·29 ± 4%, $P < 0·005$) and the total number of elastic fibres per mm^2 of dermis (3440 ± 895, $P < 0·005$) in the non-smokers. The distributions of the diameter of the fibres indicated that in the smokers these were broader than in the non-smokers (Fig. 2). No significant difference in the fibre length was found.

The mean relative areas occupied by the vertical thin fibres perpendicular to the dermal–epidermal junction and the arciform elastic fibre network were not significantly different between smokers and non-smokers, being 1·8 ± 0·5% in the smokers and 1·9 ± 0·8% in the non-smokers. The mean dermal thickness was also similar for both smokers (1389 ± 698 μm) and for the non-smokers (1577 ± 315 μm).

Immunohistochemical results

All the dermal elastic fibres were labelled with anti-elastin, anti-P amyloid component and HB8 antibodies. The results were similar with all the antibodies. There was no difference between the superficial elastic network of smokers and non-smokers. The papillary elastic fibres of the two groups showed large variation from one individual to another and from one dermal papilla to another. These vertical elastic fibre bundles could be clearly visualized both in controls and in the smokers with the anti-elastin antibodies. The deeper elastic tissue of smokers had a modified appearance when compared to controls, with numerous shortened and broad elastic fibres. These altered fibres stained less intensely with all the antibodies used (Fig. 3). In the non-smokers, fibro-

Table 1. Antibodies used for immunohistochemical techniques

	Specificity	Dilution	Source
Monoclonal antibodies			
HB8	Microfibrillar glycoproteins associated with elastic tissue	1:100	Immunotech
Polyclonal antibodies			
1	P amyloid component	1:30	Dakopatts
2	Human elastin	1:40	Institut Pasteur
3	Fibronectin	1:20	Institut Pasteur

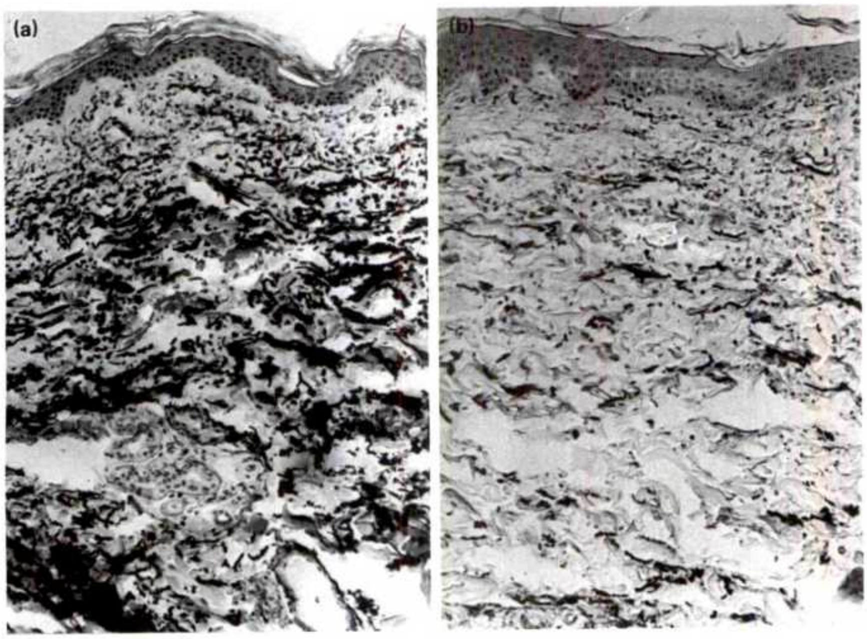

Figure 1. Dermal elastic tissue of (a) smoker and (b) non-smoker (orcein × 16).

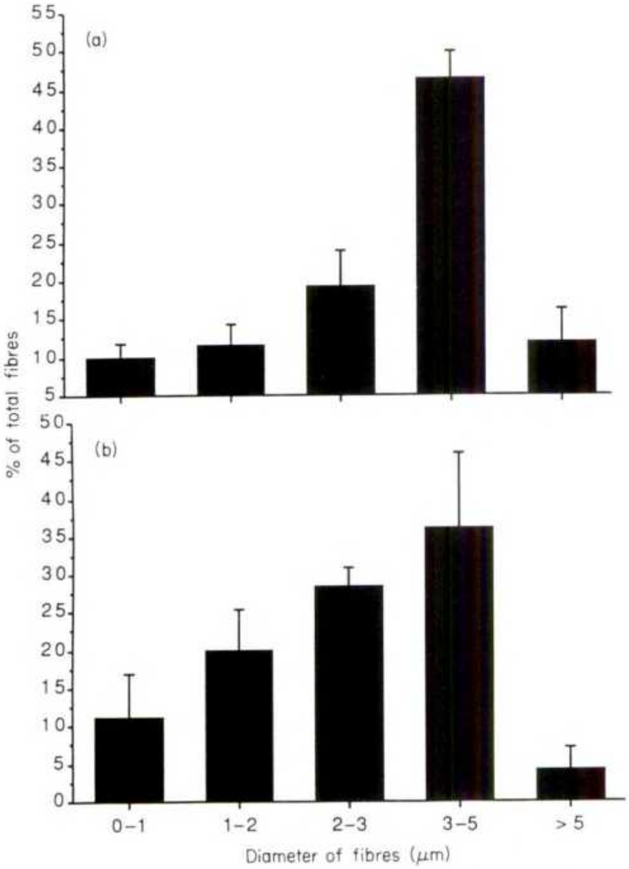

Figure 2. Distribution of the diameters of the dermal elastic fibres in (a) smokers and (b) non-smokers. The percentage of the broadest fibres (3–5 μm) is higher among smokers than non-smokers.

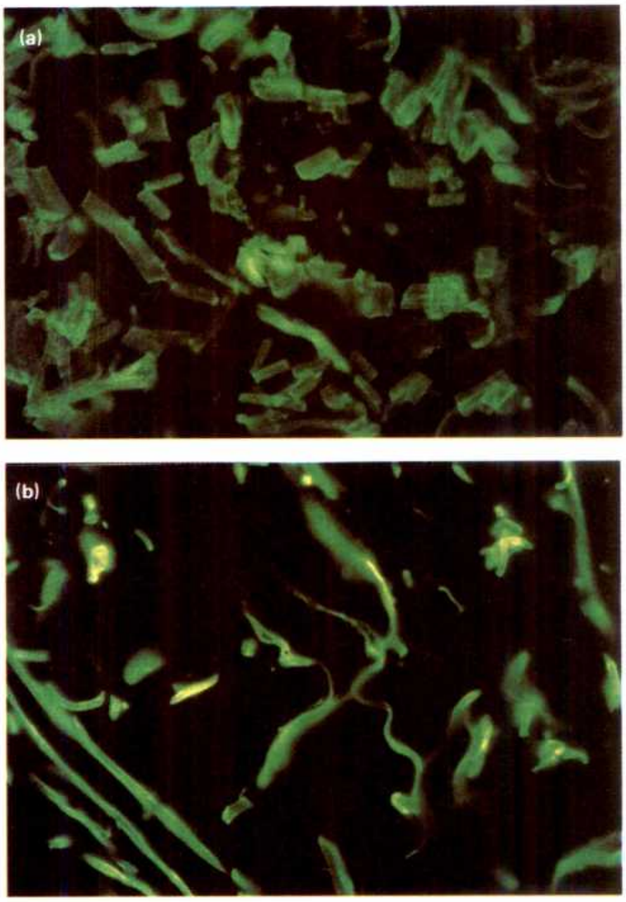

Figure 3. Mid- and reticular dermis elastic fibres in (a) smokers and (b) non-smokers. Anti-elastin antibodies were used for the first step and an FITC-conjugated goat anti-rabbit antibody for the second step (× 61).

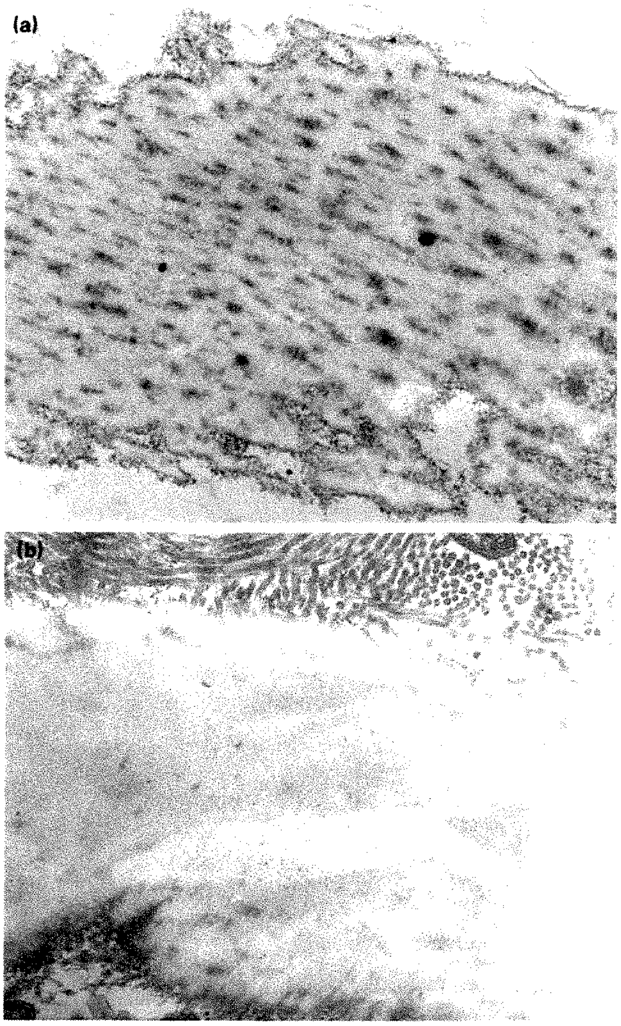

Figure 4. Electron micrograph of dermal elastic fibre from (a) smoker and (b) non-smoker showing an increased number of electron-dense regions in the fibre from the smoker (× 16,000). (Fig. 4b provided by G. Godeau, Connective Tissue Research Laboratory, Créteil).

nectin was mainly observed near the dermal–epidermal junction, forming a continuous broad band. In smokers this band was about twice as thick when compared with that in the non-smokers.

Electron microscopy

The elastin fibres in the mid- and reticular dermis of the smokers had more electron-dense regions in the amorphous fibres. These sites corresponded to the microfibril dense zones of normal elastic fibres and are normally 30–70 nm wide in longitudinal or cross-sections, and they were enlarged two- to fourfold (Fig. 4). The normal microfibrillar appearance of the dense zones[15] was replaced by dark spotted inclusions.

Discussion

We have demonstrated abnormalities in the elastic tissue of the non-sun-exposed skin of heavy cigarette smokers. To minimize the effects of photoageing, all the skin samples were taken from a sun-protected area and compared with those from sex- and age-matched non-smokers who were the controls. The total area occupied by dermal elastic fibres was increased by about 100% in the smokers compared with that found in the non-smokers. This corresponded to an increase of fibre number and fibre width and the fragmentation of the fibres was more pronounced in the smokers. Although the amount of elastic tissue was increased in the smokers, the dermal thickness was only slightly decreased. The weaker labelling of elastin and microfibrils by the appropriate antibodies in smokers as compared to the controls could indicate that both components of the elastic fibres may be abnormal. Similar results were found in solar elastosis.[16]

The appearance of these dark zones as seen on electron microscopy resembled that described in the photodamaged and senile elastotic fibres in aged skin.[17] However, unlike in solar elastosis, the papillary elastic network in the non-sun-exposed smokers' skin was normal and without a grenz zone. The abnormalities of the elastic fibres in smokers were less obvious than those seen in solar elastosis and the elastic fibres were not tangled or degenerated into amorphous masses. The elastic matrix also did not appear to be granular with a moth-eaten appearance.[18] Increased fibronectin has also been found with ageing and in conditions that are considered to be involved with ageing of skin such as diabetes and solar irradiation.[19–21] Actinic damage and heavy smoking may accelerate the changes seen in the unexposed areas and could explain the high frequency in smokers of the Favre–Racouchot syndrome (unpublished observations).

The biochemical defects of the dermal elastic tissue in smokers still remains to be determined. Previous studies have shown that cigarette smoke can increase the plasma neutrophil elastase activity[22] and the release of elastase from neutrophils[23] and can also inactivate α-1-proteinase inhibitor.[24] The plasma levels of active α-1-antitrypsin activity are decreased in smokers.[25] Elastin degradation is probably associated with elastin synthesis and in experimental emphysema induced by intratracheal instillation of pancreatic elastase, there is a marked increase in elastin synthesis within days of the injury. The enzyme which catalyses the cross-linking of elastin, lysyl oxidase, undergoes a concomitant steep rise in activity.[26] As lysyl oxidase is inhibited by some components of tobacco smoke,[27] impaired elastin cross-linking may also occur.

Although the changes in elastic fibres resemble those seen in accelerated ageing, the mid-and reticular dermis are involved, whereas the papillary dermis in the smokers is unaffected. One explanation for this difference in the localization of the changes in the dermis may be related to the vascular distribution of the toxic substances produced from smoking.

References

1 Solly S. Clinical lectures on paralysis. *Lancet* 1856; **ii**: 641–3.

2 Allen HB, Johnson BL, Diamond SM. Smoker's wrinkles. *J Am Med Assoc* 1973; **225**: 1067–9.

3 Daniell HW. Smoker's wrinkles: a study in the epidemiology of 'crow's-feet'. *Ann Intern Med* 1971; **25**: 873–80.

4 Daniell HW. Smoker's wrinkles. *J Am Med Assoc* 1973; **226**: 789.

5 Model D. Smoker's face. An underrated clinical sign? *Br Med J* 1985; **291**: 1760–2.

6 Weiss W. Smoker's wrinkles. *J Am Med Assoc* 1973; **226**: 788.

7 Godeau G, Robert AM. Use of pycnogenols and the histological staining of elastic fibres and their quantification in several tissue by automated image analysis. In: *Elastin and Elastases* (L Robert, W Hornebeck, Eds). Boca Raton: CRC Press Inc, 1989; **1**: 7–19.

8 Serra J. *Image Analysis and Mathematical Morphology.* New York: Academic Press, 1982.

9 Francès C, Branchet MC, Boisnic S *et al.* Elastic fibres in normal human skin. Variations with age: a morphometric analysis. *Arch Gerontol Geriatr* 1990; **10**: 57–67.

10 Branchet MC, Boisnic S, Francès C, Robert AM. Skin thickness changes in normal aging skin. *Gerontology* 1990; **36**: 28–35.

11 Breathnach SM, Melrose SM, Bhogal B *et al.* Immunohistochemical studies of amyloid P component in disorders of cutaneous elastic tissue. *Br J Dermatol* 1982; **107**: 443–52.

12 Schmitt D, Chignol MC, Brochier J, Thivolet J. HB8: a monoclonal antibody recognizing elastic fibre miroflbrils. *Br J Dermatol* 1986; **114**: 661–5.

13 Fyrand O. Studies of fibronectin in the skin. Indirect immunofluorescence studies in normal human skin. *Br J Dermatol* 1979; **101**: 263–70.

14 Lethias C, Hartman DJ, Masmejean M *et al.* Ultrastructural immunolocalization of elastic fibres in rat blood vessels using the protein A-gold technique. *J Histochem Cytochem* 1987; **35**: 15–21.

15 Kadar A. Ultrastructural properties of elastin. In: *Elastin and Elastases* (Robert L, Hornebeck W, eds) Vol. 1. Boca Raton: CRC Press Inc, 1989; 31–45.

16 Mera SL, Lovell CR, Russell Jones R, Davies JD. Elastic fibres in normal and sun-damaged skin: an immunohistochemical study. *Br J Dermatol* 1987; **117**: 21–7.

17 Lavker RM. Structural alterations in exposed and unexposed aged skin. *J Invest Dermatol* 1979; **73**: 59–66.

18 Braverman IM, Fonferko E. Studies in cutaneous aging: I. The elastic fibre network. *J Invest Dermatol* 1982; **78**: 434–43.

19 Phan-Thanh L, Robert L, Derouette JC, Labat-Robert J. Increased biosynthesis and processing of fibronectin in fibroblasts from diabetic mice. *Proc Natl Acad Sci USA* 1987; **84**: 1911–5.

20 Boyer B, Kern P, Fourtanier A, Labat-Robert J. Age-dependent variations of the biosyntheses of fibronectin and fibrous collagen in mouse skin. *Exp Gerontol* 1990; (in press).

21 Boyer B, Fourtanier A, Kern P, Labat-Robert J. UVA- and UVB-induced changes in collagen and fibronectin biosynthesis in the skin of hairless mice. *J. Invest Dermatol* 1990; (in press).

22 Weitz JI, Crowley KA, Landman SL *et al.* Increased neutrophil elastase activity in cigarette smokers. *Ann Intern Med* 1987; **107**: 680–2.

23 Blue ML, Janoff A. Possible mechanisms of emphysema in cigarette smokers: release of elastase from human polymorphonuclear leukocytes by cigarette smoke condensate *in vitro. Am Rev Respir Dis* 1978; **117**: 317–25.

24 Beatty K, Matheson J, Travis J. Kinetics and chemical evidence for the inability of oxidized alpha-1-proteinase inhibitor to protect lung elastin from elastolytic degradation. *Hoppe Seylers Z Physiol Chem* 1984; **365**: 731–6.

25 Hornebeck W, Solheihac JM, Velebny V, Robert L. On the influence of substrate (elastin) in elastase-α1-antitrypsin interactions. *Pathol Biol* 1985; **33**: 281–5.

26 Osman M, Kaldany RRJ, Cantor JO *et al.* Stimulation of lung lysyl oxidase activity in hamsters with elastase-induced emphysema. *Am Rev Respir Dis* 1985; **131**: 169–70.

27 Laurent P, Janoff A, Kagan HM. Cigarette smoke blocks cross-linking of elastin *in vitro. Am Rev Respir Dis* 1983; **127**: 189–94.

British Journal of Dermatology (1991) **125**, 48–52.

ADONIS 000709639100163Q

In-vivo epidermal nuclear reactions: a selective process

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Accepted for publication 30 January 1991

Summary Epidermal *in-vivo* nuclear reactions of IgG occur primarily in patients with mixed connective tissue disease or systemic lupus erythematosus and have been associated with high titres of circulating antibodies to ribonucleoprotein (RNP). This study was carried out to examine whether these epidermal nuclear reactions are true or simply an excision artefact. We observed the epidermal nuclear reactions for IgG only and not for other immunoglobulins in both *in-vivo* and *in-vitro* organ-culture studies, despite the presence of antinuclear antibodies (ANA) of all immunoglobulin classes. The association of the *in-vitro* epidermal nuclear reactions with serum RNP antibodies, although not absolute was statistically significant. The absorption of the serum with extractable nuclear antigen (ENA) preparation diminished the nuclear reactivity on tissue explants. In addition, the penetration of ANA into the nuclei of skin explants was both time and temperature dependent and was inhibited by sodium azide and by oligomycin. We conclude that the epidermal nuclear staining reactions observed by direct immunofluorescence on skin biopsies is selective and that the penetration of IgG into the epidermal cell nuclei is an active process and not an artefact.

Epidermal nuclear staining for IgG is seen on biopsies of 90% of patients with mixed connective tissue disease (MCTD), and 17% of patients with systemic lupus erythematosus (SLE).[1–8] The *in-vivo* nuclear reactions occur in both clinically uninvolved and lesional skin and in most cases give a speckled staining pattern by direct immunofluorescence (IF). These nuclear reactions have been associated with high titres of serum antibodies to extractable nuclear antigens (ENA), especially to ribonucleoprotein (RNP).[1–4] The immunoglobulin class associated with the epidermal nuclear reactions is primarily IgG, although rare cases of IgA or IgM in association with IgG[5,7,8] have been reported. There has been controversy as to whether the nuclear IgG reaction of skin biopsies is truly an *in-vivo* phenomenon[6,8–10] or whether the nuclear reaction occurs during processing of the biopsy specimen and is an artefact.[10–13]

The purpose of our study was to investigate any differences in the immunoglobulin class of antinuclear antibodies (ANA) in the sera of patients with and without *in-vivo* epidermal nuclear reactions, to determine the association of epidermal nuclear reactions with antibodies to RNP, and to study the kinetics and energy dependence of the nuclear reactions.

Methods

Serum

Sera from 25 patients with systemic connective tissue disease were selected for this study. Most of these patients fulfilled at least four of the American Rheumatism Association (ARA) criteria for systemic lupus erythematosus (SLE).[14] Of these, 13 patients exhibited *in-vivo* epidermal nuclear reactions in their skin biopsies. Nine patients had immune deposits in a granular pattern along the dermal–epidermal junction, of whom six were negative for *in-vivo* nuclear reactions by direct IF.

ANA and anti-nDNA determinations

Antinuclear antibodies (ANA) were detected by indirect IF on mouse kidney sections and on HEp-2 cells as previously described.[15] Anti-nDNA antibodies were detected by indirect IF on *Crithidia luciliae* (Immco Diagnostics, Buffalo, NY, U.S.A.). The FITC-labelled anti-human IgG conjugate was obtained from Immco Diag-

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nostics, anti-human IgA from Atlantic Antibodies, Scarborough, ME, U.S.A. and anti-human IgM from Kallestad Laboratories, Austin, TX, U.S.A. The protein content of the conjugates varied from 6·3 to 11·9 mg/ml, and the molar fluorescein to protein (F/P) ratio was 2:3. The readings were made with an epi-illumination fluorescence microscope (Reichert Scientific Instruments, Buffalo, NY, U.S.A.) equipped with a quartz halogen lamp and narrow band FITC filter.

Detection of ENA antibodies

A saline-soluble nuclear extract for RNP and Sm antigens was prepared from calf thymus nuclei as previously described.[16,17] The protein content was determined by the Hartree modification of the Lowry method.[18] Reference sera for RNP and Sm antibodies were obtained from the ANA Service, Department of Health and Human Services, Centers for Disease Control (CDC), Atlanta, GA, U.S.A. A monkey liver saline extract was used for the determination of SS-A (Ro) antibodies, and a calf thymus extract at high protein concentration (50 mg/ml) was used for the detection of SS-B (La) antibodies. Antibodies to RNP, Sm, SS-A (Ro) and SS-B (La) were detected by gel precipitation.[19]

Tissue-culture studies

An organ-culture model described by Michel and Ko[20] was used with some modifications. Human skin tissue was used within hours of excision. The tissue was kept in Hanks' balanced salt solution (HBSS), (M.A. Bioproducts, Walkersville, MD, U.S.A.) containing 50 U penicillin and 50 μg streptomycin per ml. A 0·5-mm layer of the skin was cut with a keratotome (Stortz Surgical Instruments, St Louis, MO, U.S.A.) and the skin sections were immediately placed into HBSS.

For each test, 0·2 ml of heat-inactivated serum was added to the appropriate well of an eight-well tray (Lab-Tek Products, Naperville, IL, U.S.A.). A small filter paper disc was placed into each well. To each well, 5 μl of reconstituted Fungizone (Gibco, Grand Island, NY, U.S.A.) in distilled water was added, so that each serum specimen tested contained 50 U penicillin, 50 μg streptomycin and 0·6 μg amphotericin B. Two 3 × 3 mm skin explants were placed into each well and incubated at 37°C for 3 h.

At the end of the incubation period, the skin explants were immediately rinsed in phosphate-buffered saline, excess buffer sponged off and the explants snap frozen in liquid nitrogen. Sections (4 μm) of the explants were cut in a cryostat and tested for nuclear reactions by direct IF.

Effect of absorption with ENA

Two sera were selected for the absorption study. One serum contained RNP antibodies (AS), and the other contained both RNP and Sm (BM). For the absorption, 100 μl serum was mixed with 100 μl ENA (14·3 mg/ml protein). The mixture was incubated for 1 h, centrifuged and the supernatant tested for reactivity on skin explants. As controls, unabsorbed sera from the same patients were used.

Effect of time and temperature

To study the effect of time and temperature, the explants were incubated for 1–60 min at 4, 20 and at 37°C.

Effect of inhibitors

To study the energy dependence of the antibody penetration in tissue cultures, oligomycin and sodium azide (Sigma Chemical Co., St Louis, MO, U.S.A.), were used at 2·5 μg and 10 μg, respectively, per 0·2 ml serum.[21] The skin sections were pre-incubated in HBSS containing the inhibitor for 5 min. They were immediately placed into the serum specimen containing the inhibitors at the

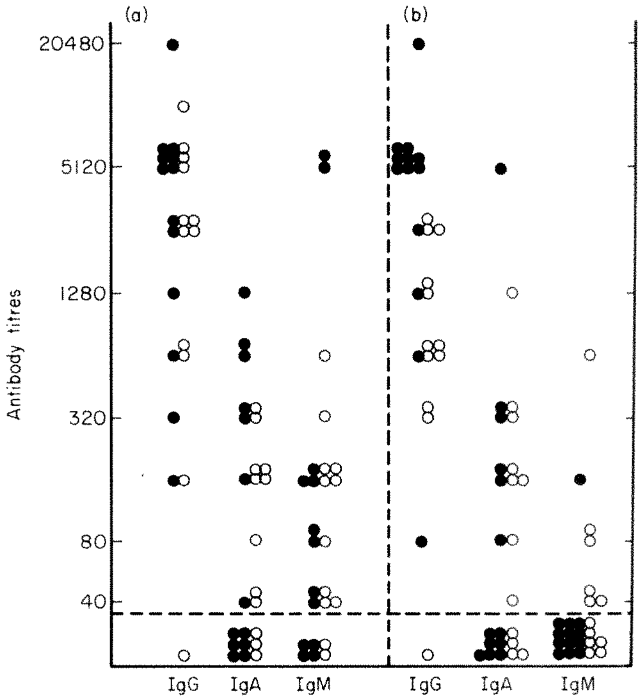

Figure 1. ANA titres in sera from patients with mixed connective tissue diseases as detected by indirect IF on: (a) mouse kidney; (b) HEp-2 cells. ●, patients positive for epidermal nuclear staining on skin biopsies; ○, patients negative for nuclear staining.

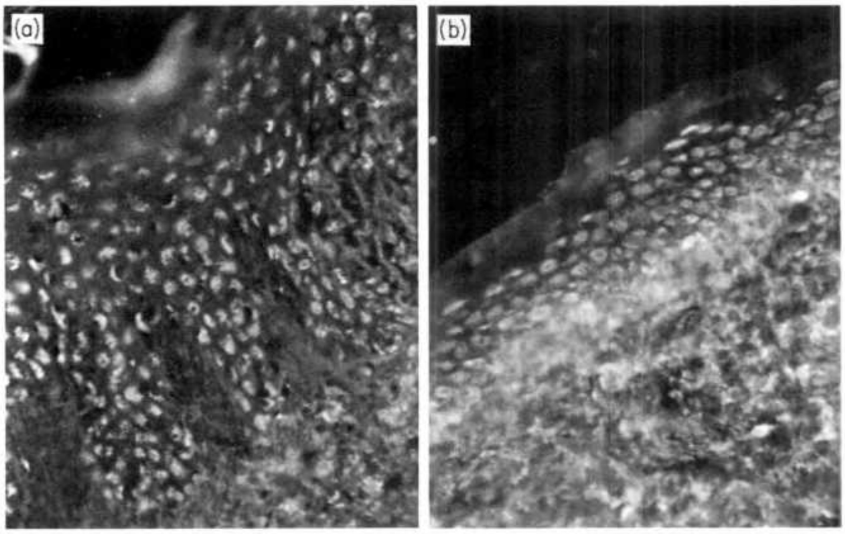

Figure 2. Direct IF staining for IgG. (a) Skin biopsy of a patient exhibiting *in-vivo* nuclear IgG deposits. (b) Normal skin explant upon incubation with the serum of the patient exhibiting positive nuclear reaction.

appropriate concentrations and incubated for 1–60 min at 37°C.

Results

Antinuclear antibodies (ANA) of varying immunoglobulin classes were present in sera from patients whether or not their skin biopsies showed nuclear reactions (Fig. 1). The epidermal nuclear reactions on skin biopsies and on the tissue explants (Fig. 2) were of the IgG class even though ANA of the other immunoglobulin classes were present in the serum. In some cases the titres of ANA of other immunoglobulin classes were equal to that of the IgG class.

Antibodies to nDNA, as determined by indirect IF on *Crithidia luciliae*, were equally distributed in both groups of patients with or without epidermal nuclear reactions.

Five of 13 patients exhibiting *in-vivo* epidermal nuclear reactions were positive for antibodies to RNP with titres ranging from 8 to 256. Three of these patients were also positive for antibodies to Sm and one patient had an unidentifiable precipitin reaction. Three of the 12 patients negative for *in-vivo* nuclear reactions had antibodies to RNP, two patients had antibodies to Sm and one had an unidentifiable precipitin line. Antibodies to SS-A (Ro) were equally distributed in both groups and were present in six patients who were positive and five who were negative for *in-vivo* epidermal nuclear reactions. Antibodies to SS-B (La) occurred in one patient of each group. Two patients with positive *in-vivo* epidermal nuclear reactions tested negative for antibodies to ENA and one had an unidentifiable reaction.

To determine whether there was any association between epidermal nuclear reactions and antibodies to RNP, the normal skin explants were cultured in the presence of sera from patients with systemic connective tissue disease. Of the 11 patients positive for epidermal nuclear reactions on skin explants, seven were positive for RNP (three out of seven also had antibodies to Sm), one was positive for antibodies to Sm alone, four had antibodies to SS-A (Ro) and two had unidentifiable reactions. In contrast, only one out of 14 sera with negative tests on skin explants had antibodies to RNP (Table 1). The association of the RNP or other ENA antibodies with epidermal nuclear staining on the explant was further shown by diminished reactions of IgG on the explants upon absorption of the sera with RNP containing ENA antigen.

To determine the effect of time, temperature and various inhibitors on IgG penetration, skin explants were cultured in the patients' sera at various time intervals. Positive reactions on skin explants were first noted at the end of 15 min of incubation. The reactions were stronger on explants incubated at 37°C compared to 4°C. Skin explants studied in the presence of oligo-

Table 1. Association of epidermal nuclear deposits in organ culture with antibodies to RNP

	Antibodies to RNP	
Epidermal nuclear reactions	Positive	Negative
Positive	7	4
Negative	1	13

Table 2. Effect of sodium azide and oligomycin on epidermal nuclear IgG reactions

Patient	Incubation time (min)	Epidermal nuclear IgG reactions by direct IF Inhibitors used:		
		None	Oligomycin	Sodium azide
BM	1	–	–	–
	5	–	w	–
	15	+	–/w	–
	30	+	–/w (focal)	–
	60	+	–	–
AS	1	–	–/w (focal)	–
	5	–	w	w
	15	+	w	w
	30	+	w	w
	60	++	–	w

++, strong positive; +, positive; w, weak positive; –, negative. BM, serum contained RNP antibodies; AS, serum contained both RNP and Sm.

mycin or sodium azide, revealed very weak epidermal nuclear reactions as compared to controls (Table 2). The presence of sodium azide either abolished or diminished the epidermal nuclear reactions.

Discussion

Direct immunofluorescence studies of skin biopsies in patients with systemic connective diseases such as SLE and MCTD yield *in-vivo* epidermal nuclear reactions rather than the most commonly observed 'lupus band' test. There has been controversy as to whether these nuclear reactions are real or laboratory artefacts. When normal human skin was cultured in the sera of these patients, we observed that most sera from patients with epidermal nuclear reactions on skin biopsies were positive on tissue explants. The nuclear staining observed in the explants was of IgG class only, even though most patients had ANA of IgG, IgA and IgM classes. In some sera, the ANA of the IgA and IgM classes had titres as high as those of the IgG class. Some investigators have reported epidermal nuclear reactions in skin or kidney biopsies involving other immunoglobulin classes,[5,22] but always in addition to IgG. Only one case with IgM epidermal nuclear staining has been reported,[8] but it was not indicated whether the IgM was a 19S or 7S immunoglobulin. We observed only IgG nuclear reactions and the patterns of fluorescence in the tissue explants were comparable to those in skin biopsies.

The association of RNP antibody with epidermal nuclear staining observed on our explant study was in agreement with previous findings.[6,23,24] Izuno[6] reported that anti-Sm antibodies did not produce nuclear staining in skin tissues *in vitro*, but few sera were tested in that study. We studied 13 sera from SLE patients with *in-vivo* epidermal nuclear staining and found that even though in general there was an association between *in vivo* epidermal staining and RNP antibodies, there were cases negative for RNP antibodies who had positive *in-vivo* nuclear reactions. One had antibodies to Sm, one to SS-A (Ro) and the other had an unidentifiable antibody. We agree with other investigators that anti-nDNA antibody is not associated with nuclear IgG deposits in the explants.[6,23] Further evidence for the selectivity of nuclear IgG reactions is provided by the fact that the nuclear staining in the explants was greatly diminished by absorption of the sera with ENA.

If the penetration of antibody into the epidermal cell nucleus is an active process and not an artefact, it should be time and temperature dependent. Our *in-vitro* studies showed an increase of IgG penetration with time and temperature. Similar time-related increases in nuclear reactions have been reported.[6,24] Active penetration by IgG is further confirmed in our study by its inhibition in the presence of sodium azide and oligomycin.

We conclude that the penetration of immunoglobulins into epidermal cell nuclei is a true phenomenon and is not an artefact, being time, temperature and energy dependent. However, the question remains whether the apparent association of nuclear IgG penetration with circulating RNP antibodies is a coincidence.

Acknowledgment

We are grateful to Mrs Gloria Griffin for her excellent editorial and typing skills. This study was supported in part by Immco Diagnostics, Buffalo, New York.

References

1 Gilliam JN, Smiley JD, Ziff M. Association of mixed connective tissue disease (MCTD) with immunoglobulin localization in epidermal nuclei of biopsies from areas of normal skin. *Clin Res* 1975, **23:** 229A.

2 Gilliam JN, Prystowsky SD. Mixed connective tissue disease syndrome. Cutaneous manifestations of patients with epidermal nuclear staining and high titre serum antibody to ribonuclease-sensitive extractable nuclear antigen. *Arch Dermatol* 1977; **113:** 583–7.

3 Shu S, Provost T, Croxdale MB *et al.* Nuclear deposits of immunoglobulins in skin of patients with systemic lupus erythematosus. *Clin Exp Immunol* 1977; **27:** 238–44.

4 Prystowsky SD, Tuffanelli DL. Speckled (particulate) epidermal nuclear IgG deposition in normal skin. *Arch Dermatol* 1978; **114:** 705–10.

5 Wells JV, Webb J, Van Deventer M *et al. In vivo* antinuclear antibodies in epithelial biopsies in SLE and other connective tissue diseases. *Clin Exp Immunol* 1979; **38:** 424–35.

6 Izuno GT. Observations on the *in vivo* reaction of antinuclear antibodies with epidermal cells. *Br J Dermatol* 1978; **98:** 391–8.

7 Anderson I, Anderson P, Elling P, Graudal H. Epidermal nuclear immunoglobulin deposits in some connective tissue diseases: Correlation with ENA antibodies. *Ann Rheum Diseases* 1983; **42:** 163–7.

8 Kallenberg CGM, deJong MCJM, Walstra TM *et al. In vivo* antinuclear antibodies (ANA) in biopsies of normal skin: Diagnostic significance and relation to serum ANA. *J Rheumatol* 1983; **10:** 733–40.

9 Alarcon-Segovia D, Ruiz-Arguelles A, Fishbein E Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. *Nature* 1978; **271:** 67–9.

10 Tuffanelli DL. Cutaneous immunopathology: Recent observations. *J Invest Dermatol* 1975; **65:** 143–53.

11 Iwatsuki K, Tagami H, Imaizumi S *et al.* The speckled epidermal nuclear immunofluorescence of mixed connective tissue disease seems to develop as an *in vitro* phenomenon. *Br J Dermatol* 1981; **107:** 653–7.

12 Barnes GW, Sullivan MA, Beutner EH, Witebsky E. *In vitro* and *in vivo* interaction of nuclear antibodies with corresponding antigens. *Experientia* 1965; **21:** 485–8.

13 Gilliam JN. The significance of cutaneous immunoglobulin deposits in lupus erythematosus and NZB/NZW F_1 hybrid mice. *J Invest Dermatol* 1975; **65:** 154–61.

14 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arth Rheum* 1982; **25:** 1271–7.

15 Beutner EH, Nisengard RJ, Kumar V. Defined immunofluorescence: Basic concepts and their application to clinical immunodermatology. In *Immunopathology of the Skin*, (Beutner EH, Chorezelski TP, Bean SF, eds), 2nd edn. New York: John Wiley and Sons, 1979; 29–75.

16 Sharp GC, Irvin WS, Tan EM *et al.* Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med* 1972; **52:** 148–59.

17 Takano M, Golden SS, Sharp GC, Agris PF. Molecular relationships between two nuclear antigens, ribonucleoprotein and Sm: Purification of active antigens and their biochemical characterization. *Biochemistry* 1981; **20:** 5929–36.

18 Hartree EF. Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry* 1972; **48:** 422–7.

19 Kumar V, Beutner EH, Dabski K *et al.* A standardized method of detecting antibodies to extractable nuclear antigens (RNP and Sm) by gel precipitation. *J Clin Lab Immunol* 1984; **15:** 163–6.

20 Michel B, Ko CS. An organ culture model for the study of pemphigus acantholysis. *Br J Dermatol* 1977; **96:** 295–302.

21 Schreiner GF, Unanue ER. Calcium-sensitive modulation of Ig capping: Evidence supporting a cytoplasmic control of ligand-receptor complexes. *J Exp Med* 1976; **143:** 15–31.

22 McCoy RC. Nuclear localization of immunoglobulins in renal biopsies of patients with lupus nephritis. *Am J Path* 1972; **68:** 469–76.

23 Claudy AL, Salandre J. Evaluation of the fixation of anti-RNP Fab-IgG on the nucleus of living cells. *J Invest Dermatol* 1980, **74:** 446.

24 Galoppin L, Saurat JH. *In vitro* study of the binding of antiribonucleoprotein antibodies to the nucleus of isolated living keratinocytes. *J Invest Dermatol* 1981; **76:** 264–7.

British Journal of Dermatology (1991) 125, 53–55. ADONIS 0007096391001645

Etretinate and visual function: a 1-year follow-up study

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Accepted for publication 28 January 1991

Summary Concern has been expressed in the literature that the drug etretinate may be potentially retinotoxic. Four patients are reported who have received long-term treatment with etretinate and who have undergone detailed evaluation for the development of visual dysfunction over a 1-year period. Despite prolonged treatment and high total doses no evidence of ocular toxicity attributable to the drug was found in any of the patients.

For many years it has been recognized that vitamin A and retinoic acid play an important part in keratinization and this has led in the past to their therapeutic use in dyskeratotic disorders. More recently synthetic retinoids, notably etretinate and isotretinoin, have been widely used for dyskeratotic disorders and, in the case of isotretinoin, acne vulgaris.

Recent reports have suggested that the use of etretinate may be accompanied by adverse ophthalmic side-effects, manifested initially by electrophysiological disturbance.[1,2] In order to determine whether the long-term administration of etretinate at recommended standard dosage is accompanied by visual or electrophysiological dysfunction, we have evaluated four patients who have received this drug for a number of years.

Methods

Four patients who were on long-term treatment with etretinate were each examined on two occasions with an interval of at least 1 year. The details of these patients are summarized in Table 1. Visual acuity was determined using the Snellen chart at 6 m, the patients wearing spectacle correction for distance if required. The visual fields were assessed using the Tübingen perimeter. Both the peripheral and central visual fields were charted and a careful search was made for the development of relative scotomata. Macular function was examined by performing central static visual field assessment across the 0–180° meridian. Colour vision was measured directly using the Farnsworth–Munsell 100 Hue test in three patients. One patient, who was known to suffer from congenital red–green dyschromatopsia, was not evaluated by this method. Electroretinogram (ERG) recordings were obtained as follows. Pupil dilatation was obtained with 1% tropicamide and corneal anaesthesia achieved with topical 0·4% benoxinate (Minims, Smith and Nephew). Gold foil electrodes (Carter and Hogg Electrodes) were positioned together with silver/silver chloride skin electrodes on both temples and the central forehead (ground electrode). Skin electrode resistance was less than 2 kΩ in all cases. Corneal responses were referenced to the ipsilateral skin contact and were amplified and passed to the analogue-to-digital convertor of the recording computer.

Light- and dark-adapted recordings were taken from both eyes simultaneously under both photopic and scotopic conditions in order to provide indices of cone and rod function, respectively. A flash stimulator (Medelec OS5; intensity setting 4) provided a diffuse circular stimulus field of 40°. Signal amplitudes were averaged from eight recordings and peak-to-peak excursions from a-wave to b-wave determined. Responses greater than 30 μv for photopic recordings and 150 μv were judged as normal for our laboratory.

The results of these investigations were compared with our normal range and the paired results for each patient were assessed for the development of any change during the 1-year period which had elapsed between examinations.

Results

The corrected visual acuities of all four patients remained unchanged in both eyes at 6/9 or better.

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Patient	Diagnosis	Duration of treatment with etretinate	Total dose of etretinate (g) received by each patient
1	Ectodermal dysplasia	2 years	14·695
2	Psoriasis	6 years, 2 months	67·280
3	Erythrokeratoderma variabillis	6 years, 5 months	88·025
4	Psoriasis	4 years	139·855

Table 1. Details of the conditions and the treatment given to each of the four patients studied

Visual field examination on Patients 1–3 was entirely normal for both eyes on both occasions. Patient 4 demonstrated bilateral non-progressive paracentral scotomata which had been a sequel to a bilateral toxic optic neuropathy sustained 3 years previously as a consequence of methotrexate therapy and which had been treated with folic acid.

With regard to colour vision, Patient 1 demonstrated bilateral congenital non-progressive red–green dyschromatopsia. Patients 2–4 gave error scores for both eyes which were within the range of normal and which had not significantly changed within 1 year.

The scotopic (rod mediated) ERG was normal for both eyes on both examinations for Patients 1 and 2. Patients 3 and 4 both showed a 10% reduction in the normal amplitude for scotopic recordings which did not change on successive recordings. None of the patients developed any abnormality in the photopic (cone mediated) electroretinograms.

Thus, none of the patients showed any evidence of progressive visual dysfunction. Patient 4 was the only individual to have slightly subnormal visual acuities at 6/9 in each eye, with small bilateral relative paracentral scotomata. This patient had been seen previously in the eye department. He had been treated with methotrexate prior to commencing etretinate therapy and the two treatments had been continued concurrently for 3 years when he developed reduced vision in 1985. At that time the visual acuities were reduced to 1/60 right and 6/60 left. He was found to have bilateral paracentral scotomata, non-specific colour vision abnormalities, normal electroretinograms but abnormal visual evoked poten-

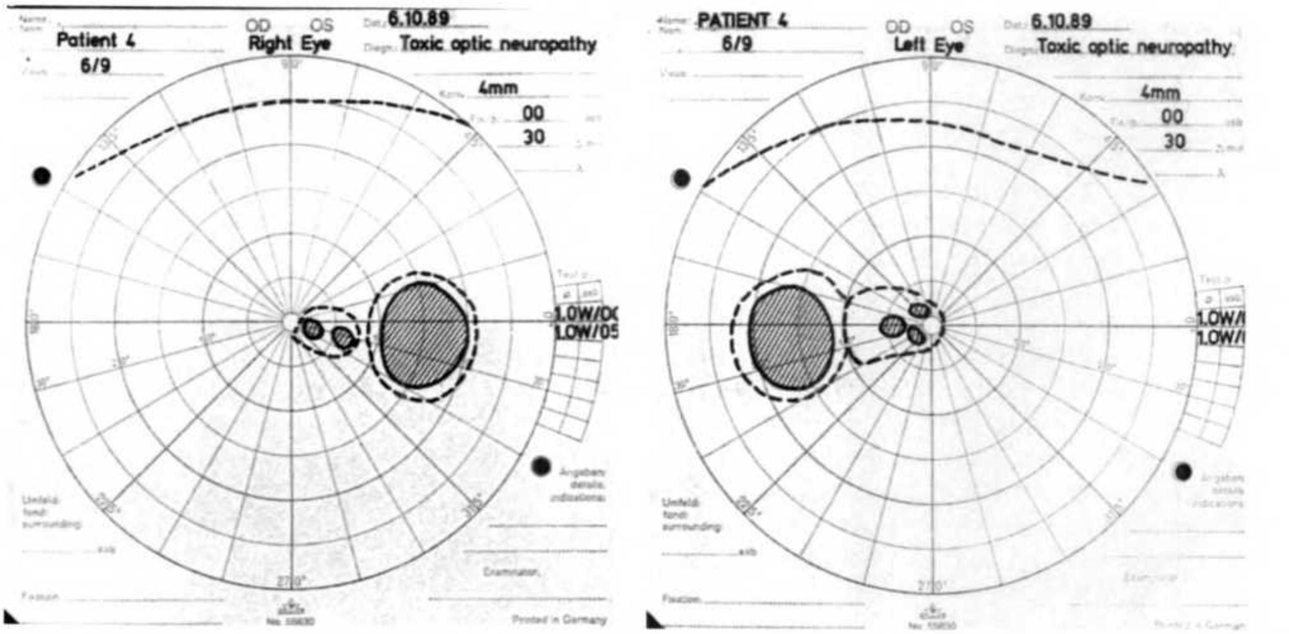

Figure 1. Central visual fields plotted on the Tübingen perimeter for Patient 4, 3 years after functional recovery from presumed methotrexate-induced toxic optic neuropathy.

tials with reduced amplitudes in both eyes and a prolonged latency for the right eye. A presumptive diagnosis of toxic optic neuropathy due to methotrexate was made and the drug was discontinued. He was treated with vitamin B_{12} and oral folate, and within 6 months the visual acuities had recovered to 6/9 in both eyes with recovery of normal colour vision. The scotomata shrank in size but remained as small areas of relative visual loss just temporal to fixation (Fig. 1). Treatment with etretinate was continued throughout the period of recovery, and for a subsequent 4 years, until the present time.

Discussion

Over the past decade etretinate has been widely used for the treatment of resistant psoriasis, Darier's disease and other dyskeratotic problems. The major contraindication to etretinate use is its teratogenicity, particularly in view of its long half-life once in the body. Other undesirable side-effects include cutaneous problems such as cheilitis and retinoid dermatitis, and elevation of the serum lipids.

Several reports have appeared in the literature regarding the ophthalmological toxicity of isotretinoin (13-*cis* retinoic acid).[3-7] More recently, Weber *et al.*[1] reported four patients who had received etretinate of whom one had reduced scotopic ERG amplitude. All four of their patients had a defective colour vision which was predominantly a blue–yellow defect. Retinal toxicity due to etretinate was presumed to be the cause of these defects. Brown and Grattan[2,3] have reported a single patient who demonstrated a fall in ERG amplitude over a 4-month period.

Because etretinate is used in the long-term control of hyperkeratotic disorders and is administered to children, such reports give rise to considerable concern. In contrast to these published reports, however, we have not been able to demonstrate any convincing evidence of progressive visual or electrophysiological dysfunction in the four patients we have studied, despite the high cumulative doses and the long duration of treatment. In particular, we did not reproduce the fairly gross defects in colour vision reported by Weber *et al.*[1] This does not, however, exclude the development of such defects in the longer term. Moreover, it is possible that the slight reduction in scotopic ERG amplitude observed in Patients 3 and 4 may have been related to antecedent treatment, although it has shown no progression over the year of follow-up.

Patient 4 is of particular interest. It is well recognized that folic acid deficiency may culminate in the development of centrocaecal scotomata as a result of optic nerve dysfunction. In this patient, both folic acid levels and vitamin B_{12} levels were normal at the time of presentation with visual symptoms. However, it was presumed that the folic acid antagonistic effect of methotrexate was responsible for the damage to the optic nerves and therefore the drug was stopped and high doses of vitamin B_{12} and folate given. Despite continuing to receive etretinate a rapid recovery of visual function in this patient was observed.

References

1 Weber U, Melink B, Goerz G, Michaelis L Abnormal retinal function associated with long-term etretinate? *Lancet* 1988; i: 235–6.

2 Brown RD, Gratten CEH. Etretinate and vision. *Lancet* 1988; i: 585–6.

3 Brown RD, Gratten CEH. Visual toxicity of synthetic retinoids. *Br J Ophthalmol* 1989; 73: 286–8.

4 Milson J, Jones DH, King K, Cunliffe WJ Ophthalmological effects of 13-*cis*-retinoic acid therapy for acne vulgaris. *Br J Dermatol* 1982; 107: 491.

5 Ensink BW, Van Voorst Vader PC. Ophthalmological side effects of 13-*cis*-retinoid therapy. *Br J Ophthalmol* 1983; 108: 627.

6 Fraunfelder FT, La Braico JM, Meyer SM. Adverse ocular reactions possibly associated with isotretinoin. *Am J Ophthalmol* 1985; 100: 534–7.

7 Weleber RG, Denman ST, Cunningham WJ, Hanifin JM. Abnormal night vision, dark adaptation and electrophysiologic responses associated with isotretinoin therapy for acne. *Clin Res* 1985; 33: 158A.

British Journal of Dermatology (1991) 125, 56–58. ADONIS 000709639100165I

Plasma levels of 8-methoxypsoralen following oral or bath-water treatment

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Accepted for publication 28 January 1991

Summary The plasma levels of 8-methoxypsoralen (8-MOP) were determined in 18 patients on PUVA treatment for their psoriasis and in two control volunteers. Seven of the patients were on oral therapy and 13 having bath treatment. The plasma levels of 8-MOP were determined up to 6 h after treatment and varied between <10 ng/ml and 360 ng/ml for the orally treated group, and in the bath-treatment group were all <10 ng/ml.

The long-term risks associated with photochemotherapy for patients with severe psoriasis are now well established.[1] Neither 8-methoxypsoralen (8-MOP) nor long-wave ultraviolet light (UVA) irradiation alone are effective. It is believed that the concentration of 8-MOP in the skin in oral therapy is directly proportional to the serum concentration of the drug[2] and related to the success of photochemotherapy.[3,4] Although 8-MOP is administered on a mg/kg body weight basis, a wide variation in the serum levels in patients has been reported.[5,6] The peak levels in the serum usually occur more than 2 h after ingestion which is the time that irradiation with UV light is usually carried out.[7,8] There are differences between the brands of 8-MOP following ingestion both in absolute serum levels and the time for maximal serum concentrations.[9]

Topically applied 8-MOP in the form of a 0·15% emulsion has been shown to give rise to plasma levels of 8-MOP comparable to those found with oral treatment. Thus it has no advantage as regards the hazards of systemic therapy[10] and another disadvantage is patchy and persistent hyperpigmentation and an uneven phototoxic response.[11] This has led to the use of topical 8-MOP mainly for localized lesions of psoriasis either in clothed areas or on the palms and soles. The majority of psoralen-bath studies have used trioxsalen (4,5′,8-trimethylpsoralen)[12–15] which is 10–30 times more photosensitizing than 8-MOP, requiring less UVA and producing a longer-lasting erythema.[16]

More recently an alternative method of topical psoralen therapy has been introduced in which the patient is immersed in a dilute solution of 8-MOP in a bath. A concentration of 8-MOP of 3·7 mg/l in the bath water has been shown to be effective with fewer side-effects and only a small amount of UVA being required for treatment.[16] We have compared the levels in the plasma of 8-MOP following oral administration and after bath treatment.

Methods

Patients

Thirteen males and seven females were studied having given informed consent (Table 1). Eighteen patients had extensive plaque psoriasis that covered more than 40% of their body surface area. Two normal controls were also included in this study. The patients were randomly allocated to receive either oral or bath PUVA treatment.

Treatment

All the participants in the study were advised to follow their normal diet and those on oral PUVA treatment had a small amount of food with their 8-MOP to offset nausea

Table 1. Detail of patients receiving 8-MOP by bath-water or oral delivery plus ultraviolet A phototherapy (PUVA)

	Bath-water delivery	Oral therapy
No of patients	13	7
Male	8	5
Female	5	2
Age range (years)	38–63	38–61
Mean age (years)	49·3	46·1

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that may be induced by taking the drug. The UVA-irradiation was performed 2 h after oral therapy and immediately following bath treatment.

The bath solutions were prepared by diluting 33 ml of a 1·12%-bath lotion of 8-MOP (Crawford Chemicals, Stony Stratford, Milton Keynes, U.K.) in 140 l of body-temperature bath water, resulting in a final concentration of 8-MOP of 2·6 mg/l. The subjects were immersed for 5 min in the bath and then patted dry and following UVA-irradiation had a shower of clean water to remove residual 8-MOP. The 8-MOP (Geroxalen, Gerot Pharmaceutical, Vienna, Austria) given orally was in a dosage of 0·41–0·63 mg/kg.

Analysis

All the solvents used for extraction and chromatographic analysis were analytical reagent grades. The 8-MOP was provided by Crawford Chemicals and the 5-methoxypsoralen used as an internal standard in each analysis obtained from Sigma Chemical Company, Poole, Dorset, U.K.

Blood samples were obtained before treatment and at 0·5, 1, 2, 4 and 6 h after ingestion of 8-MOP or following bath treatment. The plasma levels were determined using high-pressure liquid chromatography (HPLC).

Results

The plasma concentrations of 8-MOP following oral or bath treatment in the patients are shown in Tables 2 and 3.

Discussion

Our study confirms previous reports[5,6] that orally administered 8-MOP had a variable absorption rate and, as found in two of our cases, there was no rise in the plasma levels. Such a variation with a low plasma level of oral 8-MOP could account for a poor response to treatment.

Table 3. Plasma concentration (ng/ml) of 8-MOP at various time intervals after bath-water treatment

Patient	Sex	Plasma concentration of 8-MOP Pre-bath	0·5 h	1 h	2 h	4 h	6 h
S.C.	M	<10	<10	<10	<10	<10	<10
G.F.	M	<10	<10	<10	<10	<10	<10
S.T.	F	<10	<10	<10	<10	<10	<10
B.J.	M	<10	<10	<10	<10	<10	<10
B.U.	F	<10	<10	<10	<10	<10	<10
V.R.	F	<10	<10	<10	<10	<10	<10
P.W.	M	<10	<10	<10	<10	<10	<10
F.Q.	M	<10	<10	<10	<10	<10	<10
T.H.	M	<10	<10	<10	<10	<10	<10
M.B.	F	<10	<10	<10	<10	<10	<10
G.M.	M	<10	<10	<10	<10	<10	<10
H.A.	F	<10	<10	<10	<10	<10	<10
I.L.	M	<10	<10	<10	<10	<10	<10

The incidence of side-effects associated with oral PUVA is reported as 32·4% having erythema, 25·6% pruritus and 13·5% nausea.[1] In one study 15% of patients on bath PUVA treatment complained of pruritus and 35% erythema, but none of the patients had any other short-term side-effects.[16]

Information is available on the plasma concentrations of trioxsalen following bath treatment,[13,14] but there is no data on the plasma levels using commercially available 8-MOP bath lotions. The very low levels of 8-MOP found in the plasma following bath treatment indicate that very little psoralen is absorbed through the skin.

A comparison of bath PUVA between units is difficult due to the lack of standardization of a number of parameters. The selection of patients for PUVA varies

Table 2. 8-MOP dose (mg/kg body weight) and plasma concentration (ng/ml) of 8-MOP at various time intervals after oral intake

Patient	Sex	8-MOP dose	Plasma concentration of 8-MOP Pre-dose	0·5 h	1 h	2 h	4 h	6 h
S.T.	F	0·56	<10	<10	94	82	40	<10
R.S.	M	0·41	<10	<10	<10	<10	<10	<10
R.C.	M	0·57	<10	<10	<10	230	120	23
R.D.	F	0·58	<10	<10	<10	<10	<10	<10
G.A.	M	0·63	<10	<10	<10	91	360	82
N.P.	M	0·50	<10	<10	21	95	<10	<10
S.G.	M	0·60	<10	<10	<10	91	250	57

between centres and there is variation in the bath lotions used and the treatment regimes adopted. Although in two studies[16,17] a bath water concentration of 3·67 mg/l of psoralen was used, we have adopted a bath water concentration of 2·64 mg/l. Also, we have adopted a shorter bath immersion time of 5 min which we found to be effective.

In conclusion, the 8-MOP plasma levels obtained in our study following oral treatment are comparable to those found in other studies,[8] but the plasma level following bath delivery of 8-MOP was significantly lower. Younger patients embarking on potentially long-term treatment or those who have responded poorly to oral 8-MOP therapy, either because of gastrointestinal intolerance or impaired absorption, are likely to benefit from topical bath-water treatment.[10]

Acknowledgments

This study was supported by a grant from Crawford Chemicals. We thank Mr D.Campbell, Mrs M.Skelton and Mrs J.Fox for technical assistance and Mrs M.Howarth for secretarial help.

References

1 Wolff K. Side-effects of psoralen photochemotherapy. *Br J Dermatol* 1990; 122 (Suppl. 36): 117–25.
2 Wulf HC, Hart J. Distribution of tritium-labelled 8-methoxypsoralen in the rat, studied by whole body autoradiography. *Acta Derm Venereol (Stockh)* 1979; **59**: 97–103
3 Ljunggren B, Carter DM, Albert J, Reid T. Plasma levels of 8-methoxypsoralen determined by high-pressure liquid chromatography in psoriatic patients ingesting drugs from two manufacturers. *J Invest Dermatol* 1980; **74**: 59–62.
4 Wagner G, Hofmann C, Busch U *et al.* 8-MOP plasma levels in PUVA problem cases with psoriasis. *Br J Dermatol* 1979; **101**: 285–92.
5 Andersen KE, Menne T, Gammeltoft M *et al.* Pharmacokinetic and clinical comparison of two 8-methoxypsoralen brands. *Arch Dermatol Res* 1980; **268**: 23–9.
6 Herfst MJ, Koot-Gronsveld EAM, de Wolff FA. Serum levels of 8-methoxypsoralen in psoriasis patients using a new fluorodensitometric method. *Arch Dermatol Res* 1978; **262**: 1–6.
7 Van Weelden H, Van Deen-De Vries CG, Leertouwer TC, Van der Leun JC The sensitivity of human skin to UV-A as a function of time after oral administration of 8-MOP. *Arch Dermatol Res* 1980; **268**: 315–17.
8 Steiner I, Prey T, Gschnait F *et al.* Serum level profiles of 8-methoxypsoralen after oral administration. *Arch Dermatol Res* 1977, **259**: 299–301.
9 Menne T, Andersen KE, Larsen E, Solgaard P. Pharmacokinetic comparison of seven 8-methoxypsoralen brands. *Acta Derm Venereol (Stockh)* 1981; **61**: 137–40
10 Neild VS, Scott LV. Plasma levels of 8-methoxypsoralen in psoriatic patients receiving topical 8-methoxypsoralen. *Br J Dermatol* 1982; **106**: 199–203.
11 Petrozzi JW, Kaidbey KM, Kligman AM Topical methoxsalen and blacklight in the treatment of psoriasis. *Acta Dermatol* 1977; **113**: 292–6.
12 Vaatainen N, Hannuksela M, Karvonen J Long-term local trioxsalen photochemotherapy in psoriasis. *Dermatologica* 1981; **163**: 229–31.
13 Fischer T, Hartvig P, Bondesson U. Plasma concentrations after bath treatment and oral administration of trioxsalen *Acta Derm Venereol (Stockh)* 1980; **60**: 177–9.
14 Salo OP, Lassus A, Taskinen J. Trioxsalen bath plus UVA treatment of psoriasis. *Acta Derm Venereol (Stockh)* 1981; **61**: 551–4.
15 Fischer T, Alsins J. Treatment of psoriasis with trioxsalen baths and dysprosium lamps. *Acta Derm Venereol (Stockh)* 1976; **56**: 383–90
16 Lowe NJ, Weingarten D, Bourget T, Moy LS. PUVA therapy for psoriasis: Comparison of oral and bath-water delivery of 8-methoxypsoralen. *J Am Acad Dermatol* 1980; **3**: 406–14.
17 Duhra P, Hodgson C, Ilchyshyn A, Porter DI. Bath PUVA for psoriasis· the West Midlands experience. *Br J Dermatol* 1990, **123**: (Suppl. 37): 42

British Journal of Dermatology (1991) **125**, 59–61. ADONIS 000709639100166V

The influence of the menstrual cycle and pregnancy on atopic dermatitis

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Accepted for publication 28 January 1991

Summary The prevalence of a premenstrual deterioration in the symptoms of atopic dermatitis, as determined by a postal questionnaire completed by 150 women, was 33%. There was a significant association between a premenstrual worsening of atopic dermatitis and the presence of the symptoms of the premenstrual syndrome ($P<0{\cdot}002$). Pregnancy had an adverse effect on atopic dermatitis in the majority of cases (52%), usually starting in the first 20 weeks of gestation, although an appreciable proportion of women (24%) had improved during their pregnancy.

The clinical activity of certain cutaneous disorders is known to be influenced by hormonal factors in women,[1–3] but the influence of the menstrual cycle on the clinical expression of atopic dermatitis has received little attention. We have noted that a number of female patients with atopic dermatitis have spontaneously reported a worsening of their symptoms in relation to menstruation. The aim of this study was to determine the prevalence of a cyclical variation in atopic dermatitis, by means of a postal questionnaire.

Methods

Patients

The study population consisted of 204 women, aged 15–45 years, selected from the departmental diagnostic index covering the previous 5 years. All fulfilled the criteria of Hanifin and Rajka[4] for atopic dermatitis.

Questionnaire

An eight-page questionnaire was sent by post to each subject. Details of their personal medical history and relevant family history were requested. Each patient was asked to state the times, if any, at which their skin was at its best and worst in relation to menstruation. They were given five possible time responses: 2 weeks and 1 week prior to menstruation, the week of menstruation and 1 and 2 weeks following menstruation.

Patients were asked to rate the symptoms of the premenstrual syndrome using a 0–5 scale, for the weeks before, during and following menstruation. The mean scores for three physical symptoms (abdominal bloating, breast tenderness and food craving) and three mood symptoms (anger, depression and irritability) were determined. A mean reduction of two scale points following menstruation has been used to define the population with the premenstrual syndrome.[5]

Patients were asked to give details of pregnancies, and to state whether the activity of their dermatitis had changed during confinement. If a change had been noted, the stage in pregnancy at which this occurred was recorded. Details concerning regular oral contraception were also obtained.

Analysis

Data were analysed using contingency tables and significance was determined using the χ^2 test.

Results

Completed questionnaires were received from 156 women (response rate 76·6%). Six replies were excluded from the analysis because of either incomplete data or a history of amenorrhoea or hysterectomy. Of the remaining 150 women, 33% had noticed a deterioration in their skin symptoms immediately before or during menstruation, and improvement in the week following menstruation was usual. A further 10% had noticed a deterioration at another time point. Seven per cent could remember a cyclical variation in symptoms but could not remember a specific time point. No cyclical variation was reported in the remaining 50%. Any variation was generally consistent and occurred with most cycles.

Fifty women (33%) had completed at least one pregnancy with a total of 88 full-term pregnancies. Twenty-six women (52%) reported a deterioration in their skin during pregnancy, whereas improvement was noted by 12 women (24%). There was no change during pregnancy in 12 women (24%). A relapse of atopic dermatitis during pregnancy after several years free of symptoms occurred in four women.

The stage during pregnancy at which deterioration occurred was recorded for 29 pregnancies. There was worsening of atopic dermatitis in the first 10 weeks in nine pregnancies, between 10 and 20 weeks in a further 12 pregnancies, between 20 and 30 weeks in four pregnancies and only in one pregnancy between 30 weeks and term. In three others a flare-up of the skin occurred in the puerperium.

Ninety women (60%) were taking, or had taken, an oral contraceptive preparation. A combined oestrogen/progestogen preparation was used by 72 women, a progestogen-only preparation by seven women and 11 women were using a triphasic pill. Thirty-six per cent (18 of 50) of women who had experienced a premenstrual flare of their atopic dermatitis were aware of cyclical activity of their dermatitis whilst using a combined oestrogen/progestogen preparation. Compared with the 'non-flaring population', 44% (33 of 75) of whom were using the oral contraceptive, this difference was not significant. The numbers of women using the progestogen only and the triphasic preparations were too small for their influence to be adequately assessed.

Somatic symptoms of the premenstrual syndrome occurred in 31% (46) of women and psychological symptoms in 36% (54). There was a significant association with the presence of symptoms of the premenstrual syndrome in that subgroup of women who reported premenstrual flaring of their disease ($P<0{\cdot}002$).

Discussion

The first reference to premenstrual exacerbation of atopic dermatitis was in 1929 when Muller reported that 23% of menstruating women with Besnier's prurigo had worsening of skin symptoms prior to menstruation.[6] Further studies reported the prevalence of premenstrual exacerbation of dermatitis to be between 4 and 100%.[7–9] However, the clinical data in these early papers were scant and only a small number of patients were studied. Garell was the first to examine in detail the effect of the menstrual cycle on the activity of atopic dermatitis.[10] In his group of 14 menstruating adolescents with atopic dermatitis, 11 (80%) had worsening of skin symptoms just prior to, or during, menstruation. Treatment with progesterone or oestrogen produced no significant benefit in nine of the subjects. The largest study is that of Rajka[11] who reported 27 (67%) of 40 women to have premenstrual flaring of symptoms.

Premenstrual deterioration in atopic asthma has been established as occurring in approximately one-third of patients.[12,13] Life-threatening asthma may occur premenstrually[14] which may respond to the administration of intramuscular progesterone.[15]

Approximately 40% of menstruating non-atopic women experience the premenstrual syndrome (PMS), and about 10% have severe symptoms.[16] In our patients with atopic dermatitis there was a significant association between somatic and psychological symptoms of the PMS and the premenstrual exacerbation of the disease. The possibility that the psychological symptoms of the PMS increase subjective awareness of cutaneous symptoms cannot be excluded. However, the mood changes of the PMS have been postulated to be due to an abnormality of neurotransmitters such as the endorphins,[17] and this may relate to a demonstrated hyporesponsiveness to the intradermally injected neurotransmitter substance P in atopic dermatitis, possibly due to a primary abnormal sensitivity of blood vessels and mast cells to this neurotransmitter in atopics.[18] A menstrual variation in the itch/pain threshold has been shown to occur, being lower in the luteal phase.[19] Similarly, the cutaneous erythema response to histamine varies with menstruation, being highest on the first day of menstrual flow.[20] These may be contributing factors in the premenstrual flaring of atopic dermatitis.

Autoreactivity against endogenous progesterone has been postulated to be causally associated with several dermatoses that show premenstrual flaring.[21] Chronic urticaria[22] and erythema multiforme[23] are recognized to occur cyclically, and the term 'autoimmune progesterone dermatitis' was introduced to describe an eczematous eruption that could be provoked by progesterone challenge.[24] However, as many of the reported patients appear to be atopic, it seems probable that a certain number of those with 'autoimmune progesterone dermatitis' may be examples of premenstrual flaring of atopic dermatitis.

The phenomenon of premenstrual flaring of atopic dermatitis strongly suggests an influence of the female sex hormones on the expression of the dermatitis reaction. This is supported by the recent observation that the intensity of the patch-test reaction to nickel in nickel-sensitive women varies in relation to menstruation.[25] However, studies based on postal questionnaires have obvious limitations and prospective studies are necessary to confirm that there is a hormonal modulation of the activity of atopic dermatitis in women.

References

1 Novak E. Menstruation and its disorders, reciprocal relations of menstruation and various diseases. *Gyn Obstet Monogr* 1931; **2:** 288.

2 Newman BA, Feldman FF. Adult premenstrual acne. *Arch Dermatol* 1954; **69:** 356–63

3 Alexander J O'D. The clinical aspects of dermatitis herpetiformis. In: *Dermatitis Herpetiformis.* (Alexander J O'D, ed). London: WB Saunders, 1975; 29.

4 Hanifin JM, Rajka G Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; Suppl. **92:** 44–7.

5 Sanders D, Warner P, Backstrom T, Bancroft J. Mood, sexuality, hormones and the menstrual cycle. Changes in mood and physical state: description of subjects and methods. *Psychosomatic Medicine* 1983; **45:** 487–501.

6 Muller A. Uber Blutzuckerwerte bie Hautkrankheiten. *Arch Dermatol* 1929; **157:** 639–46

7 Brunsting LA. Atopic dermatitis (disseminated neurodermatitis) of young adults. *Arch Dermatol* 1936; **34:** 935–57.

8 Pirila V. On Besnier's prurigo in Finland. *Acta Derm Venereol (Stockh)* 1950; **30:** 114–32.

9 Hellerstrom S, Lidman H. Studies of Besnier's prurigo (atopic dermatitis). *Acta Derm Venereol (Stockh)* 1956; **36:** 11–22.

10 Garell DC. Atopic dermatitis in females during adolscence. *Am J Dis Children* 1964; **107:** 350–5.

11 Rajka G. Factors influencing atopic dermatitis. In: *Atopic Dermatitis.* (Rajka G, ed). London: WB Saunders, 1975; 135–7.

12 Claude F, Allemy Vall R. Ashtma et menstruation. *Presse Medicale* 1938; **38:** 755–9.

13 Gibbs CJ, Coutts II, Lock R *et al.* Premenstrual exacerbation of asthma. *Thorax* 1984; **39:** 833–6.

14 Eliasson O, Scherzer HH Recurrent respiratory failure in premenstrual asthma. *Connecticut Medicine* 1984; **48:** 777.

15 Beynon HLC, Garbett ND, Barnes PJ. Severe premenstrual exacerbations of asthma: effect of intramuscular progesterone. *Lancet* 1988; **ii:** 370–1.

16 Coppen A, Kessel N. Menstruation and personality. *Br J Psych* 1963; **109:** 711–21.

17 Reid RL, Yen SSC. Premenstrual syndrome. *Am J Obstet Gyn* 1981; **139:** 85–104.

18 Giannetti A, Girolomoni G. Skin reactivity to neuropeptides in atopic dermatitis. *Br J Dermatol* 1989; **121:** 681–8.

19 Procacci P, Buzzelli G, Passeri I *et al.* Studies on the cutaneous pricking pain threshold in man, circadian and circatrigintan changes. *Res Clin Studies in Headache* 1972; **3:** 260.

20 McGovern JP, Smolensky MH, Reinberg A. Circadian and circamenstrual rhythmicity in cutaneous reactivity to histamine and allergenic extracts. In: *Chronobiology in Allergy and Immunology.* (McGovern JP *et al,* eds). Springfield, Illinois: Charles C. Thomas, 1977; 79–116.

21 Zondek B, Bromberg YM. Endocrine allergy. *J Allergy* 1945; **16:** 1–16.

22 Farah FS, Shbaklu Z. Autoimmune progesterone urticaria. *J Allergy Clin Immunol* 1971; **48:** 257–61.

23 Wojnarowska F, Greaves MW, Peachy RDG. Progesterone induced erythema multiforme. *J Roy Soc Med* 1985; **78:** 407–8.

24 Shelley WB, Preucel RW. Autoimmune progesterone dermatitis: cure by oophorectomy. *J Am Med Assoc* 1964; **190:** 35–8.

25 Alexander S. Patch testing and menstruation. *Lancet* 1988; **ii:** 751.

British Journal of Dermatology (1991) 125, 62–67. ADONIS 000709639100167A

Lupus erythematosus profundus with partial C4 deficiency responding to thalidomide

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Accepted for publication 2 January 1991

Summary

A female patient with disfiguring lupus erythematosus profundus (LEP) from the age of 13 years was found to have an isolated partial C4 deficiency, with reduced levels of both allotypes, C4A and C4B. A genetic basis for the hypocomplementaemia was confirmed by a family study of complement and HLA types which revealed heterozygous null alleles for C4A and C4B in the proband. Marked improvement in her cutaneous lesions occurred with thalidomide.

Case report

The patient, an 18-year-old female Caucasian, was referred by her general practitioner with a history of progressively disfiguring lesions on her face and neck. Five years previously she had developed a chronically inflamed lesion at the site of a BCG inoculation, which had taken many months to heal. Subsequently, she developed erythematous, scaly lesions on the bridge of her nose and both ear lobes. She was referred to her local hospital where a diagnosis of discoid lupus erythematosus (DLE) was made on clinical grounds and confirmed by histology. However, her condition advanced with the development of deep inflammatory lesions typical of lupus erythematosus profundus (LEP). Therapy with potent topical steroids, antimalarials, anti-tuberculous chemotherapy and oral steroids proved ineffective and she was referred to St John's Hospital for Diseases of the Skin in 1989. Examination at this time revealed indurated and scarred lesions with surrounding inflammation on both cheeks and neck (Fig. 1a). There was marked atrophy at the inoculation site of her left upper arm. The rest of the physical examination was unremarkable.

Investigations

The following laboratory investigations were normal: full blood count, biochemical profile and ESR. Antibodies to double-stranded DNA, extractable nuclear antigens and anticardiolipin were all negative. The ANA was positive at 1:40. Total protein and albumin were normal with an isolated elevated IgM of 2·7 g/l (0·5–2·0). The total haemolytic component (CH50) was normal at 85%, but there were reduced levels of total C4, C4A, and C4B, at 26·6, 27·2 and 28·4% (percentage of normal human serum pool), respectively. Serum C3, C1q, and C2 levels were normal.

Histology

The first skin biopsy was performed at presentation in 1985 and revealed a chronic inflammatory cell infiltrate around appendages and dermal blood vessels, with hydropic changes in the basal cell layer. These features were consistent with the clinical diagnosis of DLE. A repeat biopsy (Fig. 2), 2 years later, showed a lymphocytic infiltrate extending to the level of the subcutis. In addition there was fibrosis of the fat septae and extensive hyaline deposition within the fat lobules. Lymphoid aggregates, some showing germinal centre formation, were present throughout the subcutis. Occasional foci of calcification were also noted.

Immunofluorescence

Direct immunofluorescence performed on lesional skin from both biopsies demonstrated granular deposition of IgM and C3 along the basement membrane zone.

Complement levels

The serum complement components C1q, C2, C3 and C4 were measured by single radial immunodiffusion. C4A

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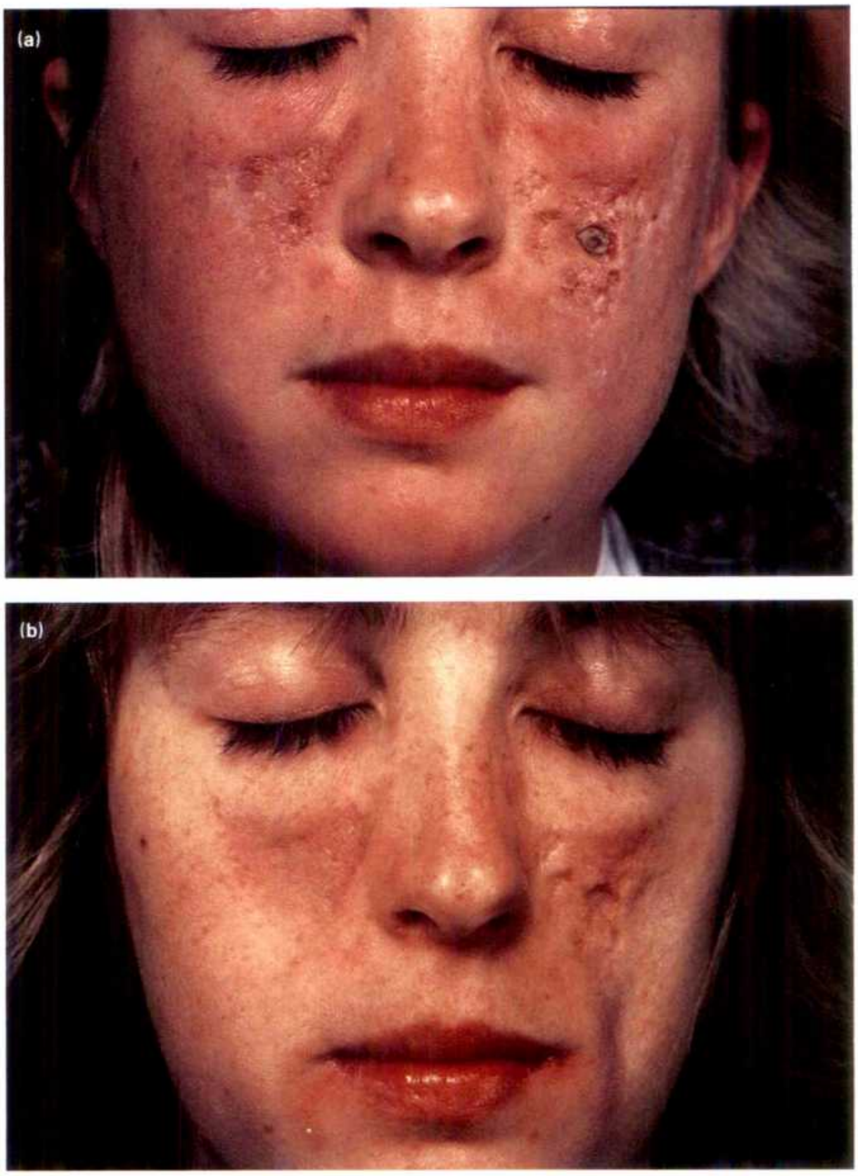

Figure 1 (a) Deep inflammatory lesions of lupus erythematosus profundus affecting both cheeks. (b) Residual scarring at sites of previous involvement following 6 months of thalidomide treatment.

and C4B levels were estimated by two-armed immunoradiometric assay (A.G.Hammond, unpublished data) using monoclonal antibodies RGD1 (anti-C4A; giff of Dr G.Giles, Royal Postgraduate Medical School), 1228 (anti-C4B; gift of Professor C.Rittner, Mainz, Germany) and T2,C5.12 (anti-C4d; gift of Professor G.D.Ross, Louisville, KY, U.S.A.).

HLA typing

This was performed by conventional two-stage microcytotoxicity testing based upon a previously described method[1] using a panel of well-characterized human antisera.

C4 allotyping

Allotyping of complement C4 proteins was performed by high-voltage agarose-gel electrophoresis.[2] To facilitate interpretation EDTA-plasma, or serum, was additionally pretreated with carboxypeptidase B (CPase B) as previously described.[3] The treatment was modified in that 18 μl of serum, or plasma, was treated with 2 μl (4·68 units) CPase in 0·1 M NaCl (Sigma: Type I from porcine pancreas) and 2 μl (0·2 units) neuraminidase (Sigma: Type VI from Cl. perfringens) in 0·2 M K_2PO_4 buffer, concurrently, overnight at 22°C in the presence of 2 μl 0·2 M EDTA. Three microlitres of sample were loaded onto the gel and the gels were run at 380 V, 100 mA

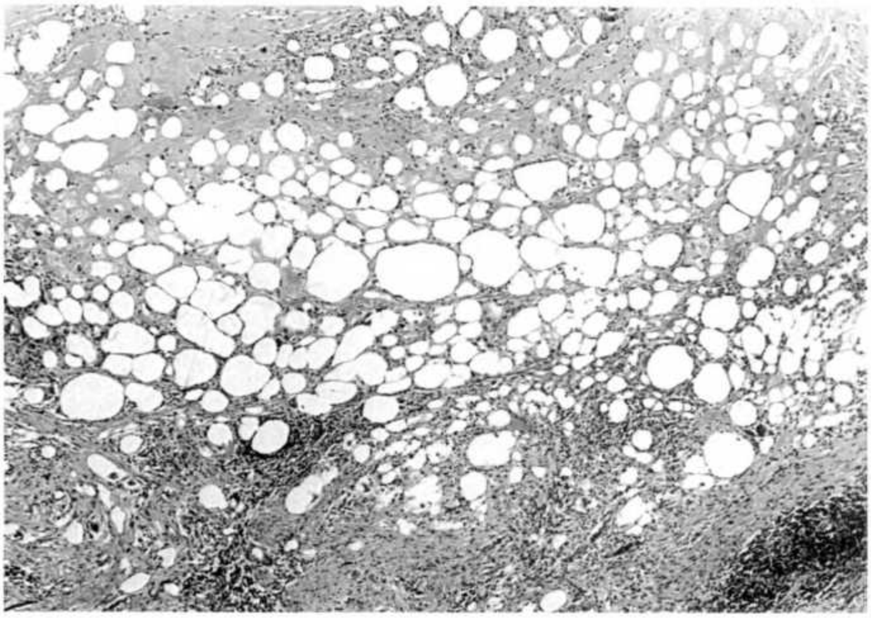

Figure 2. Skin biopsy from the cheek showing a lymphocytic panniculitis and hyaline degeneration of the fat (haematoxylin and eosin, × 150).

Table 1. Family serum complement levels*

Family member	Age (years)	C1q	C2	C3	C4	CH50
Proband	18	82	81	99	26 (C4A 17·2) (C4B 28·4)	85
Mother	51	73	85	116	87	96
Father	54	96	110	124	78	52
Brother	25	ND	ND	112	81	89
Sister	24	75	81	120	69	100
Sister	22	87	99	>140	101	104

* Percentage of NHS pool; ND, not done.

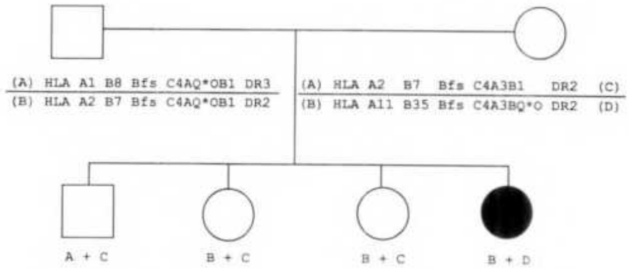
(A) HLA A1 B8 Bfs C4AQ*OB1 DR3
(B) HLA A2 B7 Bfs C4AQ*OB1 DR2
(A) HLA A2 B7 Bfs C4A3B1 DR2 (C)
(B) HLA A11 B35 Bfs C4A3BQ*O DR2 (D)
A + C
B + C
B + C
B + D

Figure 3. Family study of C4 and Factor B allotypes and HLA haplotypes. ○, female; □, male; ●, proband.

(~30 V/cm) for 3·5–4 h. Immunofixation and functional haemolytic overlay were performed as described previously.[2,4,5] Anti-human C4 complement (β1E) antibody was from Atlantic Antibodies (006-11).

C4 serum levels were reduced in the proband only, with reduction of both C4A and C4B (Table 1). The CH50 level was normal in the proband, but slightly reduced in the father. The haplotype studies suggested that the proband's C4A null allele (C4AQ*0) was inherited from the father and the C4B null allele (C4BQ*0) from the mother (Fig. 3).

Allotyping of factor B

Bf allotyping was performed by high-voltage electrophoresis of serum in 0·8% w/v agarose followed by immunofixation with anti-human factor B (Atlantic Antibodies 015-11) as previously described.[6]

Treatment and progress

Thalidomide was started at a dose of 200 mg twice daily, and after 2 months there was a noticeable reduction of the inflammatory lesions. She was subsequently maintained on 200 mg daily for a further 4 months. There was complete regression of the inflammatory lesions but she was left with residual subcutaneous atrophy at the sites of involvement (Fig. 1b). A second course of thalidomide was required, 3 months later, prior to referral to the plastic surgeons for the excision of the more disfiguring facial lesions.

Table 2. LEP cases in children under 18 years of age

Authors (year)	Age at onset	Sex	Sites affected	DLE/SLE	Treatment
Cerimele[13] (1970)	14	F	Face	−/−	HCHL
Vidal-Pich *et al.*[14] (1972)	13	F	Arm, axilla, breast	−/+	HCHL and CS
Dogliotti *et al.*[15] (1973)	16	F	Face, arms	+/−	CHL
Marks and Levene[16] (1976)	11	F	Face, arms	+/−	NS
Schnitzler *et al.*[17] (1978)	13	M	Face	−/−	CHL
	9	M	Face	−/−	CHL
Bolling[18] (1979)	16	F	Shoulder	−/−	CHL
Koransky and Esterly[19] (1981)	9	M	Face, arm	+/−	CS
Dor *et al*[20] (1982)	10½	F	Mesenteric fat	−/+	CS
Kind *et al.*[21] (1986)	4	M	Face, trunk, arms	+/−	CHL
Taieb *et al.*[22] (1986)	7	F	Face, neck, shoulder, arm	−/−	CHL and CS
Fox *et al.*[23] (1987)	3½	F	Face, back, buttocks	+/−	HCHL
	8	F	Face	+/−	HCHL and CS

LEP, lupus erythematosus profundus; DLE, discoid lupus erythematosus; SLE, systemic lupus erythematosus; +, features present; −, features absent; HCHL, hydroxychloroquine; CHL, chloroquine; CS, corticosteroids; NS, not stated.

Discussion

Lupus erythematosus profundus may develop in the absence of clinical lesions of discoid LE or systemic LE (SLE).[7] It is important to recognize this clinical subtype because the diagnosis may otherwise be missed, especially when there is no laboratory evidence of LE. The age of onset is usually in the second to sixth decade of life with a female preponderance of 2:1.[8] Clinically the subcutaneous nodules, or plaques, which are often painful, show a predilection for proximal sites, and the overlying skin may be normal or show ulceration, hyperkeratosis or atrophy.[9–11]

The histological features of hyaline degeneration, lymphoid aggregates and foci of calcification in the subcutis are characteristic of LEP.[10–12] If these hallmarks are present the diagnosis can be made, even in the absence of epidermal changes. However, if the diagnosis is in doubt direct immunofluorescence may demonstate immunoreactant deposition (the lupus band) at the basement membrane zone. Tuffanelli[11] reported positive direct immunofluorescence in the biopsies of normal-appearing skin, in three of six cases.

Our case demonstrates the typical clinical and histological features of LEP as a sequel of DLE. However, onset in childhood is rare and there have been only 13 previous reports describing LEP occurring under the age of 18 years (Table 2).[13–23] In only one case were comprehensive complement studies performed. Taieb *et al.*[22] described a 7-year-old girl who developed recurrent nodules and subcutaneous plaques on her arms, shoulders and face. Histological and immunofluorescence studies confirmed the diagnosis of LEP. There were no clinical signs of DLE and she did not fulfil the American Rheumatism Association criteria for SLE. A family study of HLA antigens and complement revealed an inherited heterozygous deficiency of C2 and C4B in the proband.

Both genetic and environmental factors appear to play a role in the pathogenesis of SLE.[24] Of the genetic factors, complement deficiency is a major determinant and deficiencies of all the early components of the classical pathway have been associated with SLE.[25] Fielder *et al.*[2] reported the importance of the presence of null alleles for C4A and C4B in determining disease susceptibility in white patients with SLE. Of the 24 patients examined, 18 were found to have null alleles at the C4A locus whilst five patients demonstrated null alleles at the C4B locus. SLE patients with C4 deficiency tend to exhibit similar clinical features with an onset in the first few years of life, marked photosensitivity, and severe Raynaud's phenomenon.[26] Discoid LE, although less commonly reported with complement deficiencies, has been described with C1 INH, C1q, C2 and partial C4B deficiencies.[27]

An important function of complement is opsonization, the coating of antibody–antigen complexes by complement to facilitate clearance by the macrophage system. This modification of immune complexes, which involves activation of the classical pathway, prevents the formation of large, insoluble immune-complex lattices.[28,29] The smaller, soluble immune complexes, formed intravascularly, may then be transported in the circulation bound to the complement receptor (CR1)[30] which is found predominantly on the surface of erythrocytes.[31]

The opsonized immune complexes are therefore delivered more readily to the macrophage system.

Studies on the handling of immune complexes by the two C4 allotypes have shown that although C4A is more effective, both C4A and C4B can inhibit immunoprecipitation. In addition, and perhaps more importantly, immune-complex binding to CR1 is much more efficient in the presence of C4A, and to a lesser degree C4B, than a control.[32] It seems reasonable, therefore, to propose that the hereditary deficiency of complement, in our case C4A and C4B, may be associated with an abnormality in the handling and solubilization of immune complexes. It may also have contributed to the relatively early age of onset.

Treatment for the majority of patients with LEP is similar to that for chronic DLE. However, as our patient developed progressive lesions on the face and neck, despite treatment with the topical steroids, hydroxychloroquine and prednisolone, she was commenced on thalidomide. The therapeutic effect of thalidomide was assessed in 60 patients with chronic DLE.[33] A marked improvement was seen in 90%, but 71% relapsed after stopping therapy, thus requiring a second, or third, course. The most important side-effect noted was a peripheral polyneuropathy and 15 patients complained of paraesthesiae and/or sensory loss. Although most of the side-effects disappeared following withdrawal of the drug, a few patients reported sensory disturbances after 1 year. Wulff *et al.*[34] recommended that thalidomide should be used on a short-term basis only, after finding that all eight of his patients receiving thalidomide for nodular prurigo developed peripheral neuropathy. In three patients followed up after withdrawal of thalidomide there was no improvement in the neuropathy. Neurological assessments should therefore be made before, and during, therapy. Our patient has been free of side-effects and repeated nerve-conduction studies have been normal.

In conclusion, this case further illustrates the importance of complement deficiencies in autoimmune disorders. We recommend that complement studies should be carried out in all patients with LEP, and in particular those starting in childhood. Our case also identifies thalidomide as a useful therapeutic option in this condition if standard treatments fail.

Acknowledgment

The publication of the colour photographs was made possible by an educational grant from Beecham Research.

References

1 Terasaki PI, Bernoco D, Park MS *et al.* Microdroplet testing for HLA-A, -B, -C, and -D antigens. *Am J Clin Pathol* 1978; **69:** 103–20.

2 Fielder AHL, Walport MJ, Batchelor JR *et al.* Family study of the major histocompatability complex in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility. *Br Med J* 1983; **286:** 425–8.

3 Sim E, Cross SJ. Phenotyping of human complement C4, a class III HLA antigen. *Biochem J* 1986; **239:** 763–7.

4 Awdeh ZL, Alper CA. Inherited structural polymorphism of the fourth component of human complement. *Proc Natl Acad Sci USA* 1980; **77:** 3576–80.

5 Zhang WJ, Kay PH, Cobain TJ, Dawkins RL. C4 allotyping on plasma or serum: application to routine laboratories. *Human Immunol* 1988; **21:** 165–71.

6 Alper CA, Boenisch T, Watson L. Genetic polymorphism in human glycine-rich beta-glycoprotein. *J Exp Med* 1972; **135:** 68–80.

7 Winkelmann RK. Panniculitis in connective tissue disease. *Arch Dermatol* 1983; **119:** 336–44.

8 Sánchez NP, Peters MS, Winkelmann RK. The histopathology of lupus erythematosus panniculitis. *J Am Acad Dermatol* 1981; **5:** 673–80.

9 Peters MS, Su WPD. Lupus erythematosus panniculitis. *Med Clin North Am* 1989; **73:** 1113–26.

10 Winkelmann RK. Panniculitis and systemic lupus erythematosus. *JAMA* 1970; **211:** 472–5.

11 Tuffanelli DL. Lupus erythematosus panniculitis. *Arch Dermatol* 1971; **103:** 231–42.

12 Fountain RB. Lupus erythematosus profundus *Br J Dermatol* 1968; **80:** 571–9.

13 Cerimele D. Contributo allo studio del lupus eritematoso profundo. *Chron Dermatol* 1970; **1:** 295–312.

14 Vidal-Pich E, Bianchi C, Bianchi O, Casala A. Lupus eritematoso profundo y calcinosis cutis. *Med Cutan Iber Lat Am* 1972; **6:** 489–94.

15 Dogliotti M, Hartdegen R, Schmaman A. Lupus erythematosus profundus: A case report on an African patient. *Dermatologica* 1973; **147:** 376–80.

16 Marks R, Levene GM. Discoid lupus erythematosus and lupus erythematosus profundus in a child. *Clin Exp Dermatol* 1976; **1:** 187–90.

17 Schnitzler L, Verret J-L, Schubert B, Ginies J-L. Le lupus érythémateux profond: Intérêt de l'étude ultrastructurale. *Ann Dermatol Venereol* 1978; **105:** 153–61.

18 Bolling R. Erythematodes profundus (Irgang-Kaposi). *Z Hautkr* 1979; **54:** 1081–2.

19 Koransky JS, Esterly NB. Lupus panniculitis (profundus). *J Paediatr* 1981; **98:** 241–4.

20 Dor AM, Kohler JL, Aubrespy P *et al.* Panniculite pseudotumorale du meso gastrosplenique. Forme inaugurale inhabituelle d'un lupus érythémateux aigu chez une fille de 10 ans. *Sem Hop Paris* 1982; **58:** 2847–50.

21 Kind P, Schreier-Rometh U, Wahn V *et al.* Lupus erythematodesus profundus. *Klin Padiat* 1986; **198:** 62–4.

22 Taieb A, Hehunstre JP, Goetz J *et al.* Lupus erythematosus panniculitis with partial genetic deficiency of C2 and C4 in a child. *Arch Dermatol* 1986; **122:** 576–82.

23 Fox JN, Klapman MH, Rowe L. Lupus profundus in children: Treatment with hydroxychloroquine. *J Am Acad Dermatol* 1987; **16:** 839–44.

24 Walport MJ, Black CM, Batchelor JR. The immunogenetics of SLE. *Clin Rheum Dis* 1982; **8:** 3–21.

25 Rynes RI. Inherited complement deficiency states and SLE. *Clin Rheum Dis* 1982; **8**: 29–47.

26 Tappeiner G, Hintner H, Scholz S *et al.* Systemic lupus erythematosus in hereditary deficiency of the fourth component of complement. *J Am Acad Dermatol* 1982; **7**: 66–79.

27 Guenther LC. Inherited disorders of complement. *J Am Acad Dermatol* 1983; **9**: 815–39.

28 Schifferli JA, Bartolotti SR, Peters DK. Inhibition of immune precipitation by complement. *Clin Exp Immunol* 1980; **42**: 387–94.

29 Schifferli JA, Ng YC, Peters DK. The role of complement and its receptor in the elimination of immune complexes. *New Engl J Med* 1986; **315**: 488–95.

30 Cornacoff JB, Hebert LA, Smead WL *et al.* Primate erythrocyte-immune complex-clearing mechanism. *J Clin Invest* 1983; **71**: 236–47.

31 Ross GD, Medof ME. Membrane complement receptors specific for bound fragments of C3. *Adv Immunol* 1985; **37**: 217–67.

32 Gatenby PA, Barbosa JE, Lachmann PJ. Differences between C4A and C4B in the handling of immune complexes: the enhancement of CR1 binding is more important than the inhibition of immunoprecipitation. *Clin Exp Immunol* 1990; **79**: 158–63.

33 Knop J, Bonsmann G, Happle R *et al.* Thalidomide in the treatment of sixty patients with chronic discoid lupus erythematosus. *Br J Dermatol* 1983; **108**: 461–6.

34 Wulff CH, Hoyer H, Asboe-Hansen G, Brodthagen H. Development of polyneuropathy during thalidomide therapy. *Br J Dermatol* 1985; **112**: 475–80.

British Journal of Dermatology (1991) **125**, 68–70. ADONIS 000709639100168N

Periarticular fibrous nodules in Lyme borreliosis

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Accepted for publication 7 January 1991

Summary Periarticular fibrous nodules have been described in patients with late-phase acrodermatitis chronica atrophicans (ACA) in the setting of Lyme borreliosis. We report the clinical and histopathological study of two patients with periarticular fibrous nodules and with Lyme borreliosis and discuss the relationship between these nodules and ACA.

Lyme borreliosis is caused by a spirochaete, *Borrelia burgdorferi*, and transmitted by ticks of the genus *Ixodes*. Its mode of clinical presentation is variable, but certain characteristic skin symptoms may appear including periarticular fibrous nodules (PFN).[1–3] We describe two patients who had PFN arising from Lyme borreliosis.

Case reports

Case 1

A 59-year-old man presented with arthralgia in the large joints and tightening of the skin of both hands, axillae and groins. His personal history was unremarkable. Clinical examination revealed that the patient had difficulty in moving his hands and fingers which appeared to be hard and stiff but with a normal skin colour. There were erythematous, violaceous, hard, scleroderma-like plaques in both axillae and groins that extended to the lumbar region. Six flesh-coloured, hard nodules 0·3 cm in diameter were seen at the interphalangeal joints of both hands (Fig. 1). The rest of the clinical examination was normal.

Laboratory tests including rheumatoid factor, muscle enzymes, ANA, anti-DNA, anti-ENA, anti-Ro, anticentromere and anti-Scl70 antibodies were all normal or negative. The VDRL and FTA tests were also negative. X-ray studies including barium examination of the oesophagus were unremarkable, as were the electrocardiogram, electromyogram and pulmonary function tests. A specimen of cerebrospinal fluid (CSF) showed no abnormality. The histology of a plaque from the groin was consistent with scleroderma and a biopsy from the right palm revealed mucin deposits and a proliferation of fibroblasts in the lower dermis. There was no hyperkeratosis, epidermal atrophy, basal liquefaction degeneration, telangiectasia or dermal mononuclear infiltrate with plasma cells. Cultures for *B. burgdorferi* from biopsy specimens yielded negative results. A biopsy from a periarticular nodule showed disorganized bundles of thickened collagen, surrounded by diffuse dermal fibrosis (Fig. 2). Indirect immunofluorescence detected antibodies to *B. burgdorferi* at IFA titres of 1/512.

Treatment with oral prednisone 80 mg/day was begun but changed to intravenous penicillin G, 3×10^6 IU/4 h for 3 weeks. The patient showed marked improvement. Twelve months later, there was still a slight induration of the hands and plaques, and the PFN persisted, but no new lesions had appeared. At that time, the IFA titre to *B. burgdorferi* had decreased to 1/128, and this remained unchanged except for an occasional decrease to 1/64.

Case 2

A 71-year-old man had a progressive tightening of the skin involving all four limbs with hard plaques over the trunk. He reported having had tick bites on several occasions which were not followed by skin lesions. He also complained of paresthesiae in the ulnar area of both forearms. Clinical examination revealed painless scleroderma-like plaques on the abdomen and lumbar region. There was a diffuse hardening of the skin with telangiectasia and dyschromia on all four limbs, but the hands and feet were unaffected. On the lateral aspects of his fingers, near the joints, there were a few hard nodules 0·3 cm in diameter with a normal skin colour (Fig. 3).

Laboratory investigations including ANA, anti-DNA, anti-ENA, anti-Ro, anti-centromere, and anti-Scl70 antibodies were normal or negative. The VDRL and FTA tests were also negative. A skin biopsy specimen from the left arm was consistent with a diagnosis of acrodermatitis chronica atrophicans (ACA). The histology of one of the periarticular nodules showed an increased amount

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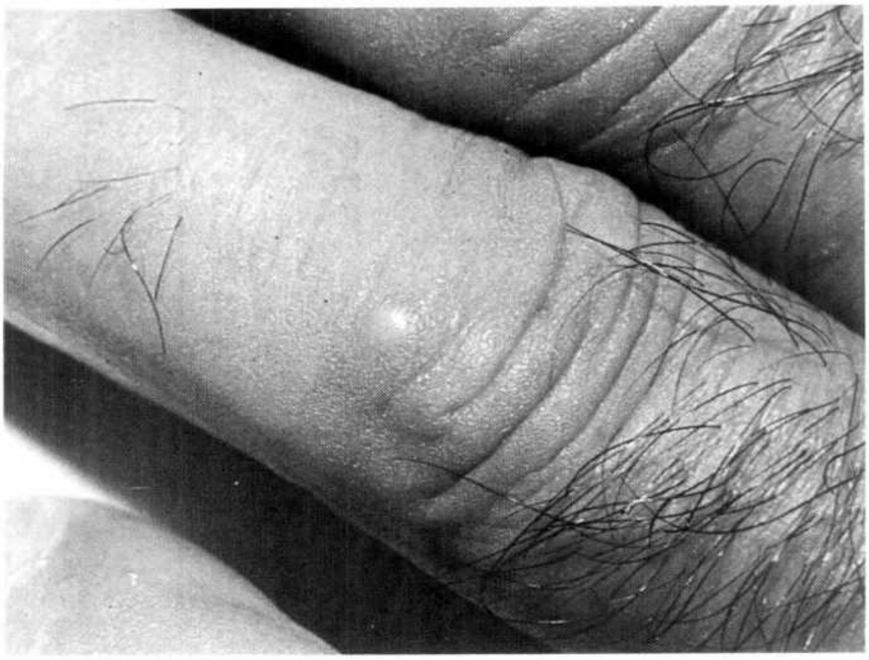

Figure 1. Case 1. Fibrous nodule on finger.

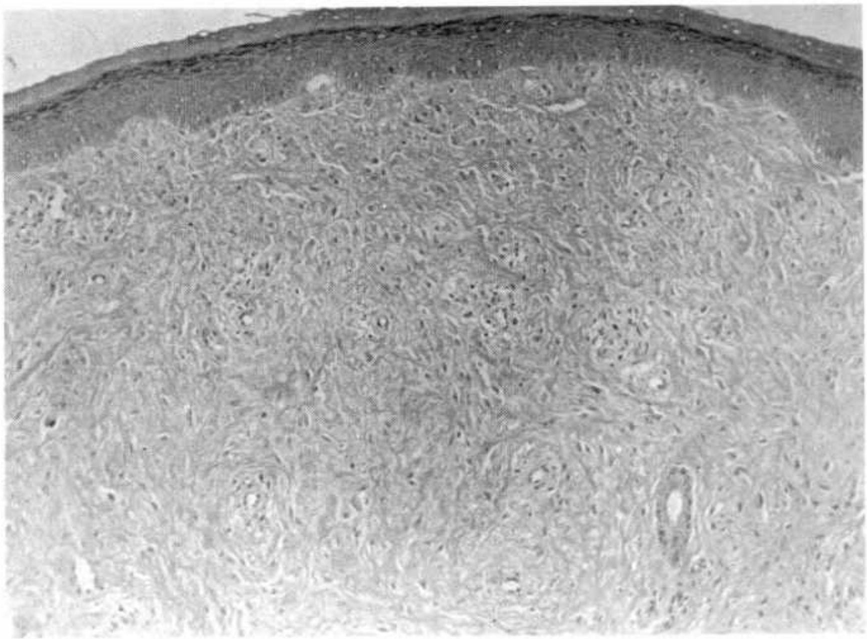

Figure 2. Case 1. Biopsy of periarticular nodule showing bundles of thickened collagen (haematoxylin and eosin, × 60).

of bundles of thick collagen in the dermis. IFA to *B. burgdorferi* was positive at titres of 1/128. An ELISA technique was performed by optic densitometry, comparing our patient's serum with serum from a patient known to have Lyme borreliosis with antibodies to *B. burgdorferi*. A ratio of 1 or more is considered positive. Indices of 1·08 and 1·2 with an interval of 3 weeks were recorded in our patient. Cultures of biopsy specimens in a modified Kelly's medium were negative.

Treatment with amoxycillin, 500 mg/6 h for 1 month induced a moderate improvement in the ACA, and the PFN completely disappeared. After treatment the ELISA test became negative, though the IFA remained at the same titre.

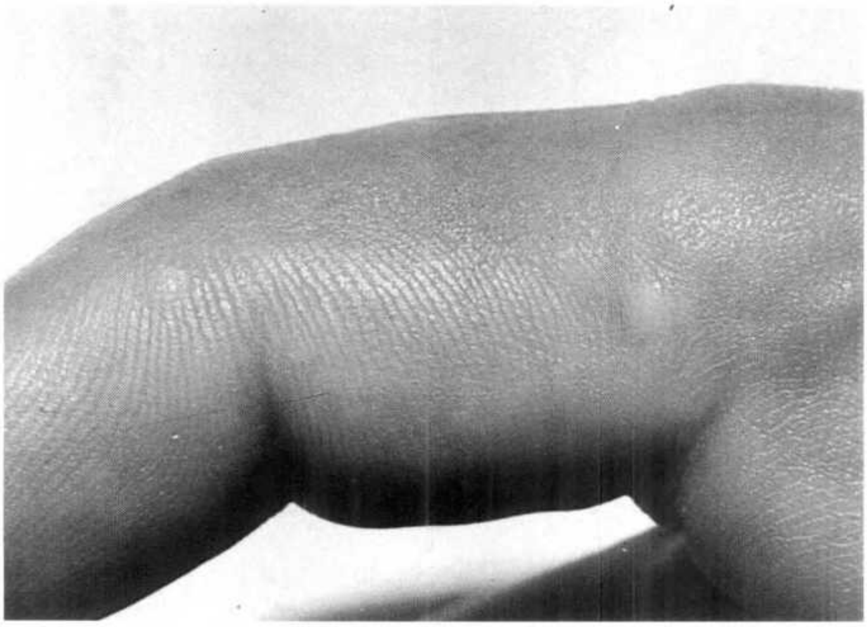

Figure 3. Case 2. Fibrous nodules on the lateral aspect of the finger.

Discussion

Both these cases were diagnosed as Lyme borreliosis from the clinical appearance of the lesions and the positive serology. Cultures for *B. burgdorferi* in our patients were negative but this spirochaete is difficult to recover from the cutaneous lesions of Lyme borreliosis.[2] The cutaneous manifestations of Lyme borreliosis include erythema chronicum migrans, lymphadenosis benigna cutis, ACA and scleroderma-like lesions.[1–3] PFN have previously been reported in patients with ACA,[4] and PFN was found in three out of nine patients with long-standing ACA.[5] Since then references to PFN have appeared in several reviews[1,2,5] but new cases have rarely been reported.[6]

One of our patients (Case 1) lacked the characteristic signs of ACA, suggesting that PFN could be a distinctive manifestation of Lyme borreliosis. However juxta-articular fibromatous nodules may appear in other spirochaetoses such as late yaws and syphilis, when they are called Lutz–Jeanselme nodules.[7,8] We believe that the presence of PFN may be of value in the diagnosis of Lyme borreliosis even in the absence of clear-cut ACA.

References

1 Burgdorf WH, Worret W-I, Schultka O. Acrodermatitis chronica atrophicans. *Int J Dermatol* 1979; **18:** 595–601.

2 Asbrink E, Hovmark A. Cutaneous manifestations in *Ixodes*-borne *Borrelia* spirochetosis. *Int J Dermatol* 1987; **26:** 215–23.

3 Duray PH, Asbrink E, Weber K. The cutaneous manifestations of human Lyme disease: A widening spectrum. In: *Advances in Dermatology* (Callen JP, Dahl MV, Golitz LE, Schachner LA, Stegman SJ, eds). Chicago: Year Book Medical Publisher, 1984: 15–21.

4 Sweitzer SE, Laymon CW. Acrodermatitis chronica atrophicans. *Arch Dermatol Syphilol* 1935; **31:** 196–212.

5 Weber K, Schierz G, Wilske B, Preac-Mursic V. European erythema migrans disease and related disorders. *Yale J Biol Med* 1984; **57:** 463–71.

6 Tazelaar DJ, Velders AJ, de Koning J, Hoogkamp-Korstanje JAA. Acrodermatitis chronica atrophicans. *Br J Dermatol* 1990; **123:** 677.

7 Gay J, Guthe T. *Treponematosis y Enfermedades Venereas*, 4th edn. Barcelona: Editorial Científico-Médica, 1969; 126–7.

8 Perine PL. Nonvenereal treponematoses: yaws, pinta, and endemic syphilis. In: *Harrison's Principles of Internal Medicine* (Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS, eds), 11th edn. New York: McGraw-Hill Book Company, 1987; 650–2.

British Journal of Dermatology (1991) **125**, 71–72 ADONIS 0007096391001692

Pellagra in a patient with an eating disorder

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Accepted for publication 16 January 1991

Summary A case of pellagra is described that occurred in a patient with an eating disorder and who presented with marked photosensitivity and diarrhoea. We found urinary 5-hydroxy-indole-acetic acid to be low and suggest that this may be a useful screening test. To our knowledge this is only the second reported case of pellagra associated with an eating disorder.

Pellagra is associated with a photosensitive dermatitis, diarrhoea, and dementia, but the full triad of symptoms occurs in only 22% of patients.[1] The mechanism of photosensitivity has not been elucidated. Skin manifestations of pellagra generally precede other symptoms, and may present as sunburn-like erythema with bullae, or more characteristically as hyperpigmentation and desquamation of sun-exposed sites.

Case report

A 19-year-old woman complained of marked photosensitivity of 10 months' duration. This began with a sunburn reaction after using a sunlamp for tanning purposes; the lamp had exploded during use. The erythema affected her face and anterior trunk, sparing the bikini regions. Blistering occurred followed by desquamation and hyperpigmentation. Subsequently, 5–10 min of exposure to the midday sun was sufficient to trigger this sunburn-like reaction in exposed sites, and sometimes also in sites covered by light clothing. Previously she had tanned easily and never burned.

Over the previous 6 months she had lost 30 kg in weight and suffered recurrent postprandial epigastric pain and vomiting and occasional diarrhoea. Her past history included asthma which lasted 1 year, readily controlled with fenoterol hydrobromide and beclamethasone diproprionate inhalers. She had previously been treated for epilepsy, but had not required anti-epileptic drugs for over 12 months. She took no other prescribed or non-proprietary medications.

On examination the patient was of normal build with erythema and slight scaling affecting the face, neck, upper limbs, and V of neck region, with sparing of the upper eyelids and those areas shaded by the chin and ears (Fig. 1). Slight hyperpigmentation and desquamation was observed on the remainder of the anterior trunk, sparing the bikini areas. Physical examination was otherwise unremarkable. Normal investigations were as follows: complete blood count, electrolytes, liver function tests, B_{12}, folate, antinuclear antibodies, porphyrin screens (urinary, faecal, serum), chest X-ray, and gastroscopy. Photopatch testing with the Trolab photoallergen series was negative. The 24-h urinary

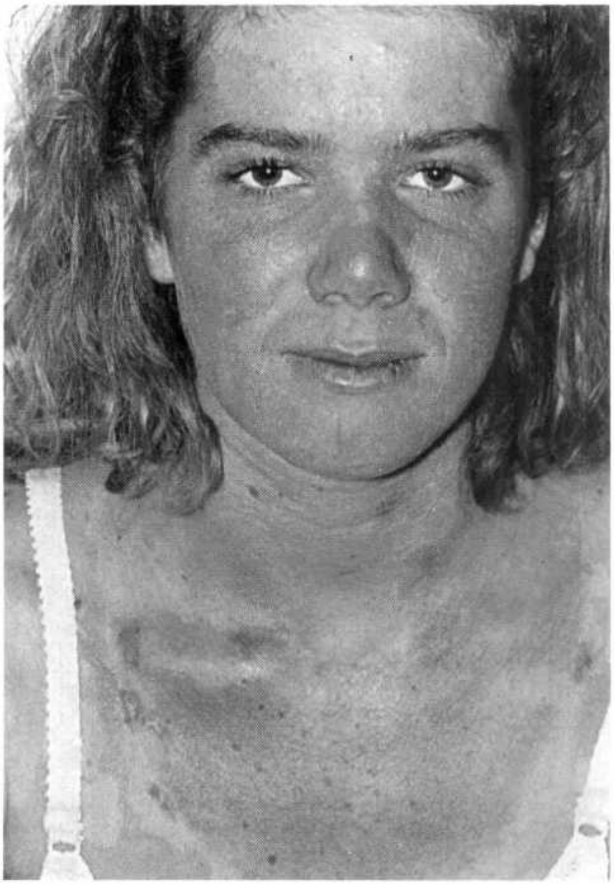

Figure 1. Erythema of photoexposed areas of the face and neck.

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5-hydroxy-indole-acetic acid (5-HIAA) was low at 2 μmol/24 h (normal: 7–37 μmol/24 h).

While an in-patient, she was noted to behave in a juvenile manner, and her food intake was negligible. Subsequent interviews with her friends confirmed an inadequate diet with a past history of anorexia nervosa. There was no current history of laxative abuse or induced vomiting, nor evidence for abnormal body image. Her usual habitus was that of obesity. A diagnosis of pellagra was made, and she was commenced on niacin 150 mg daily, and referred for psychiatric counselling and dietary advice. There was complete resolution of her symptoms within 48 h, and at follow-up several months later she remained well.

Discussion

To our knowledge there is only one previous case report of pellagra in association with anorexia nervosa.[2] Pellagra is caused by dietary deficiency of niacin or tryptophan, impaired assimilation of these nutrients in malabsorption states, or altered tryptophan metabolism. It is most commonly seen in those whose diet is inadequate due to poverty, or in alcoholics. Protein deficiency exacerbates niacin deficiency as tryptophan is used in preference to other amino acids to maintain nitrogen balance.[3] The supply of other amino acids, especially leucine and isoleucine, as well as the endocrine status of the individual are also contributary in the regulation of tryptophan metabolism.

Although chronic alcoholism is the commonest cause of pellagra in developed countries today, few alcoholics develop pellagra, even when the most impoverished cases are considered. Likewise, while certain drugs seem able to induce pellagra—notably isoniazid[4]—they do so only in a small minority of individuals receiving the drug. Because isoniazid is a structural analogue of niacin, a process of competitive inhibition may be operating. Many of the patients who develop pellagra while on isoniazid are of poor nutritional status and it is likely that the drug serves to exacerbate a latent niacin deficiency. Drug- and alcohol-induced pellagra are complex metabolic problems which are not yet fully understood.

Carcinoid tumours divert large amounts of tryptophan to serotonin synthesis resulting in niacin deficiency and occasionally pellagra. This is accompanied by increased urinary excretion of 5-HIAA, the end product of serotonin metabolism. Conversely, patients with pellagra have reduced CSF and urinary 5-HIAA,[5] possibly due to the absence of feedback inhibition of niacin on the kynurenin pathway. The low levels of urinary 5-HIAA found in our patient therefore support a diagnosis of pellagra, and this may be a useful diagnostic test in hospitals, such as ours, that are unable to provide estimation of plasma tryptophan or urinary N-methylnicotinamide. The patient's prompt response to niacin therapy confirmed this diagnosis.

Our patient was unusual in not having the cachectic appearance characteristic of those with pellagra or anorexia nervosa. However, she had lost a substantial amount of weight, and her dietary habits were grossly abnormal, being deficient in calories, protein and niacin. This case highlights the need for a high index of suspicion in making a diagnosis of pellagra.

References

1 Spivak JL, Jackson DL. Pellagra: An analysis of 18 patients and a review of the literature. *Johns Hopkins Med J* 1977; 140: 295–309.
2 Rapaport MJ. Pellagra in a patient with anorexia nervosa. *Arch Dermatol* 1985; 121: 255–7.
3 Vivian VM, Chaloupka MM, Reynolds MJ. Some aspects of tryptophan metabolism in human subjects. *J Nutr* 1958; 66: 587–98.
4 Cohen LK, George W, Smith R. Isoniazid-induced acne and pellagra. *Arch Dermatol* 1974; 109: 377–81.
5 Raghuram TC, Krishnaswamy K. Serotonin metabolism in pellagra. *Arch Neurol* 1975; 32: 708–10.

British Journal of Dermatology (1991) **125**, 73–75. ADONIS 000709639100170U

Localized pemphigoid shares the same target antigen as bullous pemphigoid

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Accepted for publication 18 January 1991

Summary A case of localized pemphigoid is described in which the lesions were only on the lower legs. Immunoblotting of the patients' serum reacted with a 240-kDa polypeptide identical to one of the bullous pemphigoid antigens. IgG1 was detected in the lesional skin and IgG1 and IgG4 in the patient's serum. These observations suggest that the immunopathological mechanisms in the blister formation in localized pemphigoid are similar to those found in more widespread bullous pemphigoid.

Localized pemphigoid is a rare, non-mucosal blistering disease.[1] It is accepted that this disease occurs in two forms, one with scarring plaque-like lesions that are located on the head and neck (Brunsting and Perry type),[2] and the other with non-scarring bullous lesions usually on the lower legs.[3] As in bullous pemphigoid (BP), most patients with localized pemphigoid have immunoglobulin and/or complement bound to the basement membrane zone (BMZ).[4] Although the disease is probably the result of an autoimmune process, the sera from patients with localized pemphigoid show intermittently circulating anti-BMZ antibodies.[4] Little information is known of the target antigen(s) at the dermal–epidermal junction that are involved in localized pemphigoid.

We report a case of localized pemphigoid in which the lesions were located solely on the lower legs and have investigated using Western immunoblotting the nature of the target antigens.

Case report

A 65-year-old woman presented with a blistering eruption which was localized solely to the lower legs and which had recurred periodically over the previous 5 months. The blisters were associated with itching. There was no previous history of trauma and there was no involvement of the skin eleswhere or of her mucous membranes. On examination there were circumscribed areas, 3–4 cm in diameter, which were pigmented with several bullae and erosions and crusts with minimal scarring on both lower legs (Fig. 1).

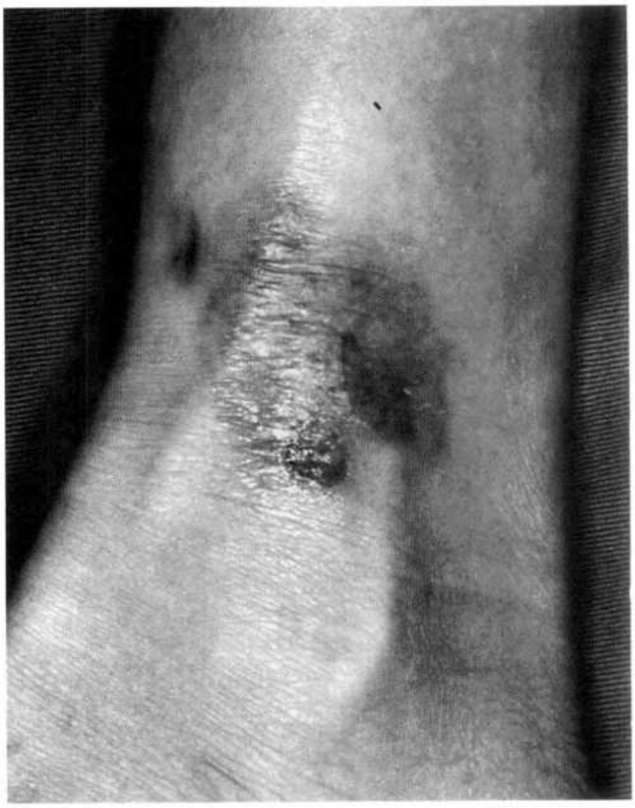

Figure 1. Bullae, crusts and hyperpigmented areas on the left lower leg.

Skin biopsy

A biopsy was taken from the margin of a blister and on histology showed a subepidermal bulla with a cellular infiltrate that contained eosinophils, polymorphonuclear leucocytes and mononuclear cells. Direct immunofluorescence performed on perilesional skin showed linear deposits of IgG, IgE and C3 at the dermal–epidermal junction zone.

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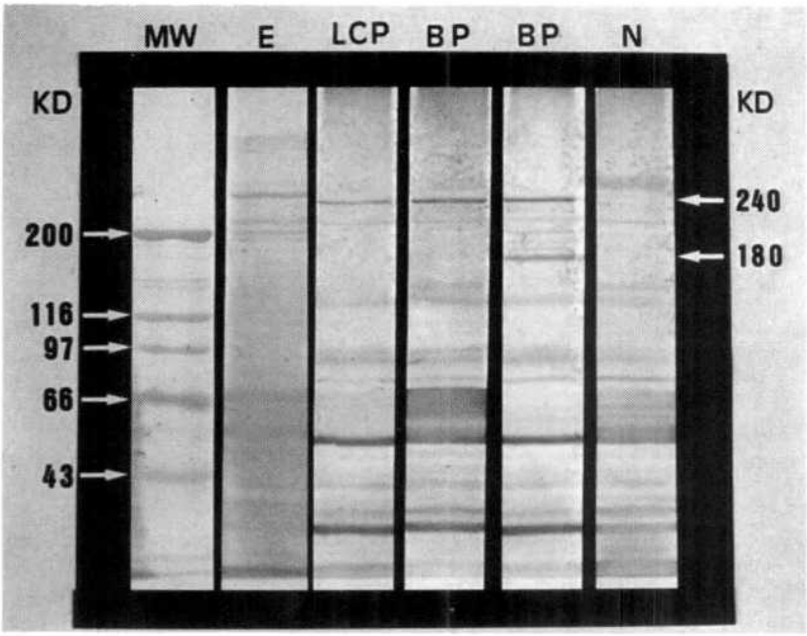

Figure 2. Results of Western immunoblotting. Lane MW: proteins of standard molcular weight. Lane E: human epidermal extract stained with Amido black. Lane LCP: serum from our patient with a 240-kDa band. Lanes BP: control sera from patients with generalized bullous pemphigoid reacting with either a 240-kDa band or 240-kDa and 180-kDa bands. Lane N: normal control serum.

Blood investigations

Circulating IgG anti-BMZ antibodies were detected at a titre of 1:5120 by indirect immunofluorescence using guinea-pig oesophagus as the substrate. Indirect assay of complement binding showed linear deposits of C3 at the dermal–epidermal junction. Indirect immunofluorescence on sodium chloride split skin showed the presence of IgG autoantibody on the epidermal side of the cleft.[5] Western blots using human epidermal extracts showed that in the patient's serum there was a band with a molecular weight of 240 kDa (Fig. 2) in lane LCP, while the sera from patients with generalized BP showed either a 240-kDa band or 240-kDa and 180-kDa bands as the BP antigens.

Other investigations

We also analysed the distribution of IgG subclasses and IgE antibodies by direct and indirect immunofluorescence and immunoblotting using monoclonal antibodies against IgG1-4 and IgE. The results are shown in Table 1.

Treatment

The patient was treated with topical applications of dexamethasone propionate 0·1% with rapid healing of the lesions with minimal scarring. Although there were recurrent lesions there was no further spread of the condition over the 23 months.

Discussion

Patients with localized pemphigoid generally have low amounts of detectable anti-basement membrane antibodies as determined by indirect immunofluorescence. This makes it difficult to characterize the target antigen and the anti-BMZ antibodies involved in the disease. However in our case, there was a high titre of circulating anti-BMZ antibody.

The histopathological findings in our case were compatible with the diagnosis of bullous pemphigoid. The results obtained from direct and indirect immunofluorescence were characteristic for this disorder. Serum from the patient reacted with a 240-kDa polypeptide, identical to one of the BP antigens as seen on Western

Table 1. Analysis of IgG subclass, IgE class and complement in biopsy specimen and serum

	IgG 1	IgG 2	IgG 3	IgG 4	IgE	C3*	C′-fixing†
DIF‡	+	−	−	−	+	+	
IIF§	+	−	−	+	+		+ +
IB¶	+	−	−	+	−		ND**

* Deposition of C3 at basement membrane zone in direct immunofluorescence.

† Complement fixing test by indirect immunofluorescence.

‡ Direct immunofluorescence.

§ Indirect immunofluorescence.

¶ Immunoblotting.

** Not done.

immunoblot.[6,7] Anti-BMZ antibodies of the subclass IgG1 were detected by direct and indirect immunofluorescence while IgG4 subclass antibody was detected only by indirect immunofluorescence. These IgG1 and IgG4 subclass antibodies also reacted with an identical 240-kDa protein on immunoblotting. Recently, several studies have described the IgG subclass distribution of BP antibodies using immunofluoresence and that IgG4 was most commonly found followed by IgG1, while IgG2 and IgG3 were only occasionally detected.[8,9]

The pathogenesis of localized pemphigoid is unknown, but our findings indicate that the blister formation is probably the same as in the more generalized bullous pemphigoid. However, this cannot explain the localized nature of the lesions, which could be due to local factors in addition to an immune reaction. Previous reports have shown that various factors can induce lesions, including the Goeckerman regime for the treatment of psoriasis, radiation therapy, ultraviolet light and mechanical trauma.[10–13] Our patient had none of these factors. It has also been reported that vascular disease may contribute to the pathogenesis of the localized pretibial lesions.[14] Our patient did have superficial varicose veins, and this may have contributed to the pathogenesis of the blisters as seen on her legs.

The serum from our patient and from two patients with generalized bullous pemphigoid all showed an identical 240-kDa band on Western blotting. This band has been identified as being the major BP antigen,[6,7] and was not labelled by the sera from normal controls. A second 180-kDa BP antigen is also recognized and this was present in the serum from one of our patients with generalized BP. In addition to these proteins, some sera from patients with BP react with multiple components of a crude epidermal extract with molecular weights of 200, 97 and 77 kDa.[7] However it is not clear which of these are pathogenic. Recently antibodies have been produced against a 230-kDa (identical to 240 kDa) BP antigen in rabbits using fusion proteins derived from fragments of a previously cloned BP cDNA.[15] These antibodies bound to the inner plaque of the hemidesmosomes of the basal cells, as shown on immunoelectron microscopy.[15] This suggests that the antibody against the 240-kDa antigen could bind to it after the injury to the basal cell membrane, and would be unable to initiate blister formation. This hypothesis supports the idea that local factors such increased hydrostatic pressure may play an important role as the initiating factor in blister formation in localized pemphigoid, with resulting antigen–antibody reaction. It may be that in localized pemphigoid there is a different set of antibodies from that seen in the more generalized type. They may also not be identified by the present techniques that are used.

References

1 Salomon RJ, Briggaman RA, Wernikoff SY, Kayne AL. Localized bullous pemphigoid. A mimic of acute contact dermatitis. *Arch Dermatol* 1987; 123: 389–92

2 Brunsting LA, Perry HO. Benign pemphigoid? A report of seven cases with chronic, scarring, herpetiform plaques about the head and neck. *Arch Dermatol* 1957; 75: 489–501.

3 Eberhartinger C, Niebauer G. Zur prognose und Therapie des Pamphigus vulgaris und ahnlicher Erkrankungen. *Hautarzt* 1961; 12: 503–8.

4 Liu H-NH, Su WPD, Rogers RS III. Clinical variants of pemphigoid. *Int J Dermatol* 1986; 25: 17–27.

5 Gammon WR, Briggaman RA, Inman AO *et al.* Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1·0 M sodium chloride-separated skin. *J Invest Dermatol* 1984; 82: 139–44.

6 Stanley JR, Woodley DT, Katz SI Identification and partial characterization of pemphigoid antigen extracted from normal human skin. *J Invest Dermatol* 1984; 82: 108–11.

7 Labib RS, Anhalt GJ, Patel HP *et al.* Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 1986; 136: 1231–5.

8 Bird P, Friedmann PS, Ling N *et al.* Subclass distribution of IgG autoantibodies in bullous pemphigoid. *J Invest Dermatol* 1986; 86: 21–5.

9 Kelly SE, Cerio R, Bhogal BS, Black MM. The distribution of IgG subclass in pemphigoid gestationis: PG factor is an IgG1 autoantibody. *J Invest Dermatol* 1989; 92: 695–8.

10 Provost TT, Maize JC, Ahmed AR *et al.* Unusual subepidermal bullous diseases with immunologic features of bullous pemphigoid. *Arch Dermatol* 1979, 115: 156–60.

11 Ernst TM, Marsch WCH. Development of localized pemphigoid following radiation treatment. *Dermatologica* 1982; 164: 73–81.

12 Cram DL, Fukuyama K. Immunohistochemistry of ultraviolet-induced pemphigus and pemphigoid lesions. *Arch Dermatol* 1972; 106: 819–24.

13 Dahl MGC, Cook LJ. Lesions induced by trauma in pemphigoid. *Br J Dermatol* 1979; 101: 469–73.

14 Person JR, Rogers RS III, Perry HO. Localized pemphigoid. *Br J Dermatol* 1976; 95: 531–4.

15 Tanaka T, Korman NJ, Shimizu H *et al.* Production of rabbit antibodies against carboxy-terminal epitopes encoded by bullous pemphigoid cDNA. *J Invest Dermatol* 1990; 94: 617–23.

British Journal of Dermatology (1991) 125, 76–78. ADONIS 0007096391001718

Dermatomyositis responding to treatment of associated toxoplasmosis

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Accepted for publication 2 February 1991

Summary We report a 59-year-old woman with severe dermatomyositis in association with high serum toxoplasma antibody titres who was successfully treated with pyrimethamine and sulphadiazine. This case supports an aetiological role for toxoplasmosis in the pathogenesis of some cases of dermatomyositis.

Polymyositis (PM) and dermatomyositis (DM) are characterized by chronic inflammation of skeletal muscle, proximal muscle weakness, elevation of serum creatine phosphokinase (CPK) and typical electromyography changes with polyphasic potentials. Histopathological examination of muscle biopsy classically shows a necrotizing inflammatory process.[1] DM is distinguished from PM by a violaceous periorbital oedema, and transient erythematous, scaly plaques over the knuckles, wrists, elbows, ankle and knees. Because the clinical course and muscle pathology are similar, DM and PM may be part of the same disease process. There have been several reports associating PM–DM with toxoplasmosis, but it is unclear whether the diseases are causally related.[2–8]

We describe a patient with severe DM associated with cutaneous necrosis and retinal microinfarcts in whom toxoplasma antibody titres were increased. Response to immunosuppressive treatment was incomplete, but resolution of the disease followed treatment with anti-toxoplasmosis therapy, pyrimethamine and sulphadiazine.

Case report

A 59-year-old West Indian woman was admitted to hospital in March 1987 with a 3-week history of progressive proximal muscle weakness, dysphagia, and bilateral periorbital oedema. She could barely walk 15 yards and was unable to raise her arms above the level of her shoulders. Examination revealed eyelid oedema, nail fold capillary thromboses, ragged cuticles, and ulcers on the dorsa of the hands (Fig. 1) and both inner thighs. Breast, pelvic and rectal examinations were normal.

Initial laboratory tests revealed normal full blood count and renal function, and an erythrocyte sedimentation rate of 35 mm/h. Other values, expressed in international units (IU), were alanine transferase 29 (normal <20), aspartate transaminase 135 (normal <18) and CPK 2150 (normal <70). Results of serum complement levels, latex fixation, antinuclear antibody and antibody to extractable nuclear antigen tests were all normal. Oesophageal motility tests showed impaired swallowing, reflux and aspiration, and electromyographic findings were consistent with a myopathic disorder. A deltoid muscle biopsy showed muscle degeneration with perivascular mononuclear cell infiltrates. Chest X-ray, sigmoidoscopy, bilateral mammogram, abdominal ultrasound and thyroid scan were normal. The diagnosis of dermatomyositis with oropharyngeal involvement was made.

She received 60 mg of prednisolone and 200 mg of azathioprine daily for 2 months. Serum enzyme activities declined but her weakness persisted. In June 1987 she was transferred to Nottingham on the same dose of azathioprine and 50 mg of prednisolone daily for outpatient follow-up. However, the patient was admitted shortly thereafter with a relapse of dermatomyositis. She was bedridden with profound muscular weakness and fever. On fundoscopy bilateral soft exudates were noted adjacent to the discs, and chorioretinal inflammatory foci adjacent to the fovea (Fig. 2). There was symptomatic improvement after increasing prednisolone to 90 mg daily for 2 weeks, but deterioration occurred on reducing the dose. Her clinical course was complicated by the development of diabetes mellitus which necessitated

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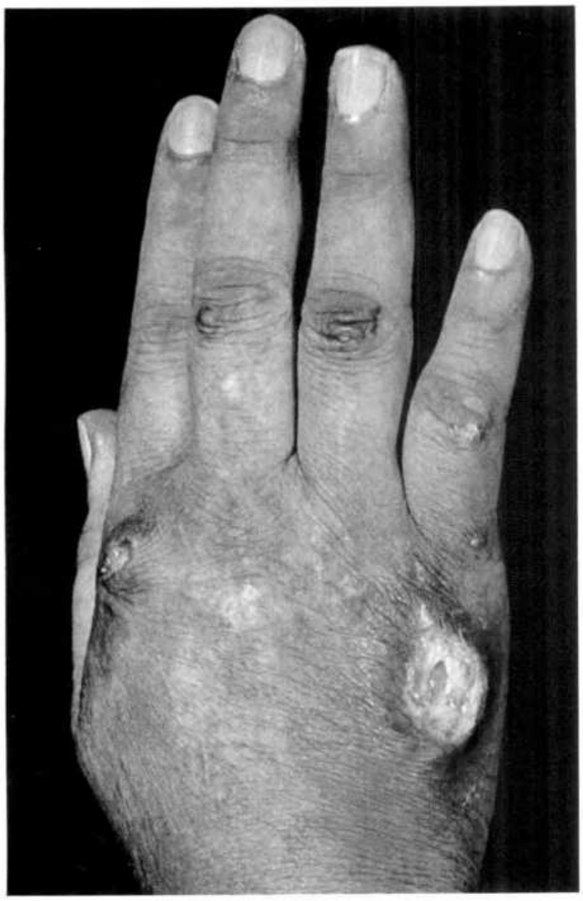

Figure 1. Ulceration of the hands in association with a papular eruption on the knuckles (Gottron's sign).

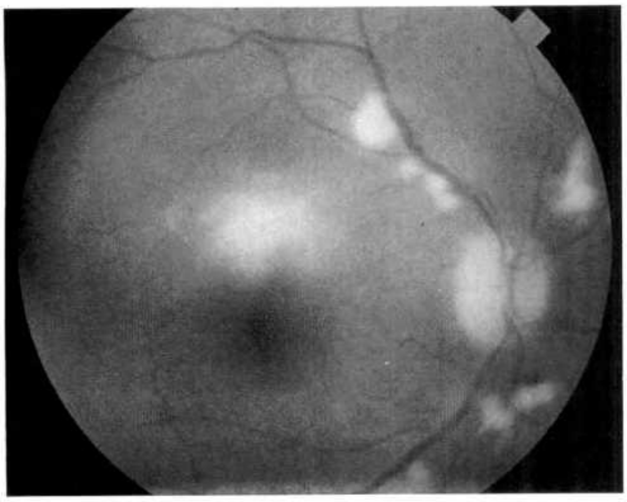

Figure 2. Fundoscopy showing chorioretinitis adjacent to fovea (centre); soft exudates are also present in relation to the optic disc.

insulin treatment, but she remained normotensive. Toxoplasma serology, which had been performed because of the known association of dermatomyositis and elevated toxoplasma antibodies, was strongly positive (latex agglutination titres > 1:2048 and the dye test 1:8192).

In view of the severity of the illness and lack of response to azathioprine and high doses of prednisolone, it was decided to treat her for toxoplasmosis using pyrimethamine 25 mg on alternate days, sulphadiazine 2 g q.d.s. and folinic acid 15 mg q.d.s. This produced prompt clinical improvement; her fever abated within a week and after 2 weeks she was able to walk. Treatment was stopped at this stage because of the development of neutropenia. Azathioprine was also discontinued, and the dose of prednisolone steadily reduced over the following 6 weeks without any recurrence of muscle weakness or rise in CPK. Intermittent courses of prednisolone have been required subsequently to control a slight ocular inflammation, but since March 1989 she has led an active life. Her toxoplasma titres have remained elevated but, despite withholding all immunosuppressive therapy, there has been no recurrence of disease activity.

Discussion

The role of toxoplasmosis in the pathogenesis of polymyositis–dermatomyositis is uncertain, although myositis is known to occur as a result of direct invasion of muscle by *Toxoplasma gondii*. It has been suggested that elevated toxoplasma antibody titres are a nonspecific immunological response to the illness, as occurs in systemic lupus erythematosus, or are the result of reactivated latent infection in an immunocompromised host.[3] However, there are reports of dermatomyositis responding to treatment of toxoplasmosis with pyrimethamine and sulphadiazine.[4–8] In all cases the toxoplasma titres have been significantly elevated and in one case toxoplasmosis was identified in the muscle biopsy.[5] These reports indicate that there may be a causal relationship between active toxoplasma infection and some cases of dermatomyositis.

An association between dermatomyositis and toxoplasmosis in our patient was based on high toxoplasma antibodies, chorioretinitis adjacent to the fovea, and recovery of dermatomyositis following treatment of toxoplasmosis with pyrimethamine and sulphadiazine. The retinal microinfarcts would be consistent with a vasculitic manifestation of dermatomyositis.[9] These appearances and the cutaneous ulceration are unusual features of dermatomyositis. Retinal microinfarcts could also represent early signs of the patient's latent diabetes mellitus, or may be related to hypertension.

Dye titres are said to be the most specific and sensitive measure of toxoplasma antibodies.[10] Active infections are associated with titres ranging from 1:1024 to 1:65,536. The titres decrease slowly and may persist for years. IgM fluorescent antibody test was negative in our case, but the prompt resolution of symptoms with pyrimethamine and sulphadiazine strongly suggests that active toxoplasmosis was responsible for the development of dermatomyositis. It is unlikely that pyrimethamine and sulphadiazine acted as immunosuppressive agents as maintenance therapy would normally be required over months or years. However, this case is remarkable in that no regular immunosuppressive therapy has been subsequently required for control of dermatomyositis over the ensuing 2 years despite the severity of the disease.

This case further supports a causal relationship between toxoplasmosis and dermatomyositis. Clinicians should be alert to this possibility and should examine the fundi and perform serological tests for toxoplasmosis. A trial of therapy with sulphadiazine and pyrimethamine may be worthwhile for severe PM or DM.

References

1 Strauss KW, Gonzalez-Buritica H, Khamashta MA, Hughes GRV. Polymyositis-dermatomyositis: a clinical review. *Postgrad Med J* 1989; **65:** 437–43.
2 Magid SK, Kagen LJ. Serologic evidence for acute toxoplasmosis in polymyositis-dermatomyositis. *Am J Med* 1983; **75:** 313–20.
3 Behan WMH, Behan PO, Draper IT, Williams H. Does toxoplasma cause polymyositis? *Acta Neuropathol* 1983; **61:** 246–52.
4 Pollock JL. Toxoplasmosis appearing to be dermatomyositis. *Arch Dermatol* 1979; **115:** 736–7.
5 Topi GC, D'Alessandro L, Catricala C, Zardi O. Dermatomyositis-like syndrome due to toxoplasma. *Br J Dermatol* 1979; **101:** 589–95.
6 Schröter HM, Sarnat HB, Matheson DS, Seland TP. Juvenile dermatomyositis induced by toxoplasmosis. *J Child Neurol* 1987; **2:** 101–4.
7 Hendrickx GFM, Verhage J, Jennekens FGI, van Knapen F. Dermatomyositis and toxoplasmosis. *Ann Neurol* 1979; **5:** 393–5.
8 Quills MR, Damjanov I. Dermatomyositis as an immunologic complication of toxoplasmosis. *Acta Neuropathol* 1982; **58:** 183–6.
9 Munro S. Fundus appearances in a case of acute dermatomyositis. *Br J Ophthal* 1959; **43:** 548–58.
10 Feldman HA. Toxoplasmosis. *N England J Med* 1968; **279:** 1370–5, 1431–7.

British Journal of Dermatology (1991) **125**, 79–80.
ADONIS 000709639100172M

Tuberculosis verrucosa cutis in a tumour-like form

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Accepted for publication 21 January 1991

Summary An unusual case of tuberculosis verrucosa cutis that presented with a tumour-like lesion on the heel is described. This responded rapidly to treatment with isoniazid.

Cutaneous tuberculosis occurs in a wide varity of forms.[1] There are however some variants such as tuberculous ulcer of the skin[2] and tumour-like forms that are reported with different descriptive terms.[3]

We describe a patient with a tumour-like lesion on his left heel with a histology suggestive for tuberculosis verrucosa cutis and which rapidly responded to treatment with isoniazid.

Case report

A 32-year-old man presented with a 1-year history of a painless warty tumour on his left heel. He came to Japan from India 3 years previously and there was no history of preceding trauma. The tumour had been gradually enlarging over several months. Previous attempts to excise the lesion were soon followed by the return of the tumour and 1 week before presentation he had swelling of his left leg up to the inguinal region.

On examination, he appeared well but with a verrucous tumour that measured 5 × 3 cm on the left heel (Fig. 1). The warty surface was covered with crusts and altered blood and there was induration of the surrounding hyperkeratotic plaque. There was left inguinal lymphadenopathy.

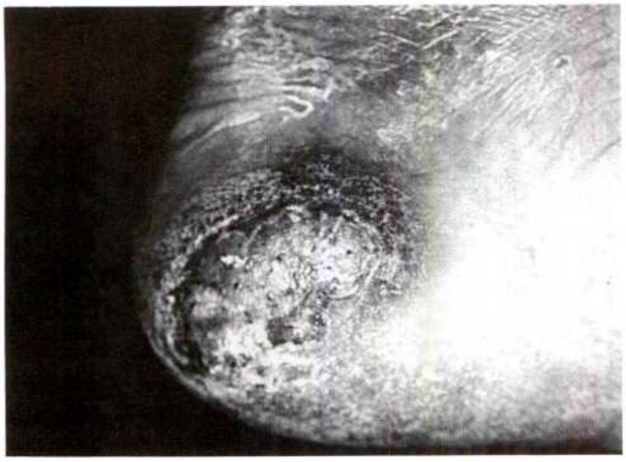

Figure 1. Verrucous tumour on the left heel.

An intradermal test with 0·05 μg purified protein derivative (PPD) was strongly positive with surrounding erythema and induration and with a blister in the centre. Culture of a portion of the biopsy did not grow any acid-fast bacilli, fungi or other microbial organisms. The histology showed hyperkeratosis and papillomatosis of the epidermis with individual cell keratinization and horn-pearl formation (Fig. 2). There was a band-like infiltrate that consisted of mononuclear cells, plasma cells and some eosinophils and there were a few foreign body giant cells in the upper dermis. No epithelioid cell granulomas with Langerhans' type giant cells could be identified. Ziehl–Neelsen staining for mycobacteria was negative and immunohistopathological staining using a monoclonal anti-human papilloma virus antibody was also negative.

Because of the strongly positive PPD reaction and the histopathological features that suggested tuberculosis verrucosa cutis, the patient was treated with isoniazid 400–600 mg daily and with local heat therapy. After 2 weeks of treatment there was a marked reduction in the size of the tumour and by day 50 it had healed completely to leave a scar and some discoloration of the skin.

Discussion

Tuberculosis verrucosa cutis is one of the most common forms of cutaneous tuberculosis in Asia. It occurs as the result of inoculation of mycobacteria into the skin of an individual who has been previously infected and who has a moderate-to-high degree of immunity.[4] Usually there are scanty bacilli in the lesions which are not detected by staining or on culture. In Europe the lesions

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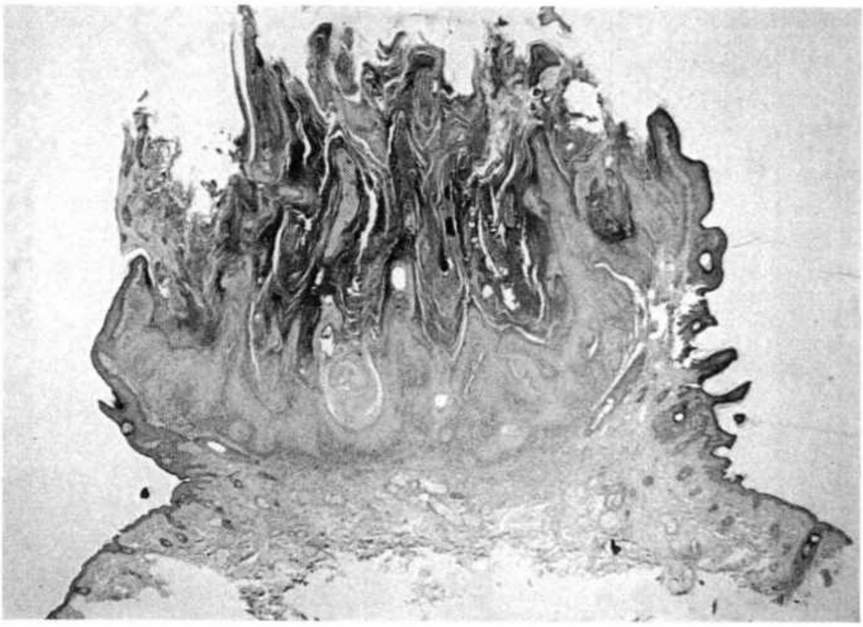

Figure 2. Hyperkeratotic lesion with eosinophilic papillomatous epidermal hyperplasia and individual cell keratinization with horn-pearl formation. A dense inflammatory cell infiltrate is present in the upper dermis (haematoxylin and eosin, × 10).

are most likely to occur on the hands, however in Asia the knees, ankles and buttocks are mainly involved.[5] Regional lymphadenitis, which occurred in our case, is rare and may be the result of a secondary pyococcal infection. The epidermis on histology shows marked papillomatosis and a pseudoepitheliomatous hyperplasia with hyperkeratosis.[6] The infiltrate in the dermis is frequently non-specific but epithelioid granulomas with giant cells may be seen.

The differential diagnosis of our case included verrucous carcinoma, deep fungal infection, atypical mycobacterial infection, tertiary syphilis, blastomycosis-like pyoderma[7] and giant verruca vulgaris. Although we were unable to demonstrate acid-fast bacilli in the lesion or on culture, the diagnosis of tuberculosis was supported by the strongly positive skin reaction to tuberculin and by the rapid response to anti-tuberculous therapy with isoniazid[8] that was combined with local heat treatment.[9]

References

1 Savin JA, Wilkinson DS. Mycobacterial infections including tuberculosis. In: *Textbook of Dermatology* (Rook A, Wilkinson DS, Ebling FJD *et al.*, eds), 4th edn. Oxford: Blackwell Scientific Publications, 1986; 798–822.

2 Lantos G, Fisher BK, Contreras M. Tuberculous ulcer of the skin. *J Am Acad Dermatol.* 1988; **19:** 1067–72.

3 Shiratori A. Tuberculosis fungosa verrucosa lymphangiectatica (Lewandowsky). *Jpn J Dermatol* 1953; **63:** 95–8.

4 Kakakhel KU, Fritsch P. Cutaneous tuberculosis. *Int J Dermatol* 1989; **28:** 355–62.

5 Wong KO, Lee KP, Chiu SF. Tuberculosis of the skin in Hong Kong (A review of 160 cases). *Br J Dermatol* 1968; **80:** 424–9.

6 Hirsh BC, Johnson WC. Pathology of granulomatous disease: Epithelioid granulomas, part I. *Int J Dermatol* 1984; **23:** 237–46.

7 Su WPD, Duncan SC, Perry HO. Blastomycosis-like pyoderma. *Arch Dermatol* 1979; **115:** 170–3.

8 Rilder HL. Tuberculosis verrucosa cutis: clinical picture and response to short-course chemotherapy. *J Am Acad Dermatol* 1988; **18:** 1367–9.

9 Tagami H, Ginoza M, Imaizumi S, Urano-Suehisa S. Successful treatment of chromoblastomycosis with topical heat therapy. *J Am Acad Dermatol* 1984; **10:** 615–19.

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Topical retinoic acid in the treatment of fine acne scarring

SIR, Tretinoin (Retin A) is a well established topical treatment for acne vulgaris. Its main mode of action is the reduction of comedogenesis and the alteration of the microclimate within involved follicles, rendering them less susceptible to *Propionibacterium acnes* colonization.[1] There is some evidence that topical vitamin A may be of benefit in the treatment of scarring, particularly for keloidal and hypertrophic lesions.[2] We report a patient in whom the daily application of tretinoin 0·05% resulted in a marked improvement in the superficial scarring secondary to acne vulgaris.

A 25-year-old Indian dentist was referred to the dermatology department for assessment and treatment of her acne scarring. She had suffered from treatment-resistant acne for many years and in 1982 she was placed on spironolactone following a diagnosis of polycystic ovaries. This drug was subsequently withdrawn and she was commenced on Diane with good results.

Examination of the skin revealed multiple ice-pick scars and shallow pits over the cheeks (Fig. 1) which were particularly obvious in view of her racial pigmentation. The patient was insistent that some treatment should be prescribed for her appearance about which she was depressed. She was therefore prescribed 0·05% Retin A cream to be applied to the unwashed affected areas at night. When reviewed 4 months later there had been a dramatic improvement in the appearance of the scarring (Fig. 2). There was no evidence of post-inflammatory hyperpigmentation.

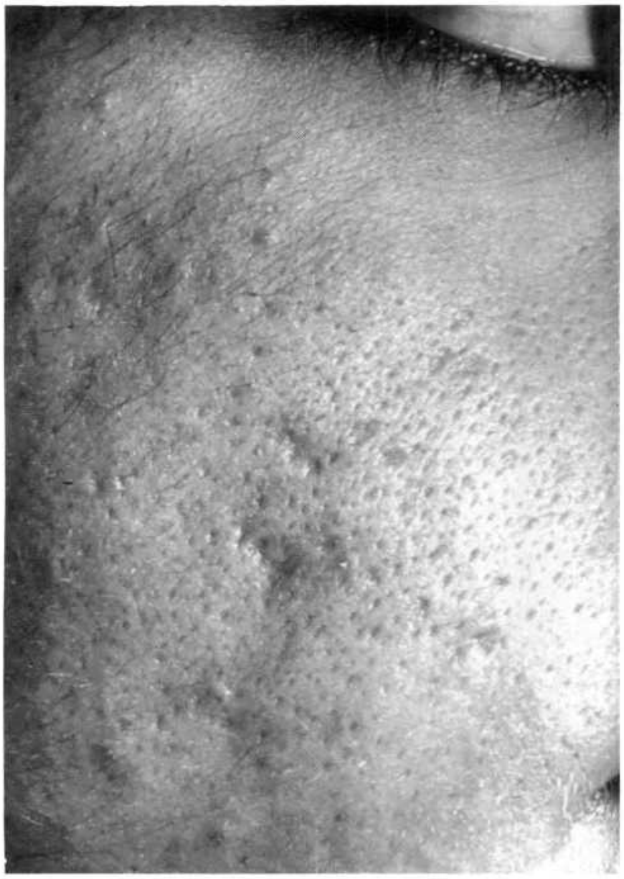

Figure 1. Fine acne scarring seen on the cheek prior to treatment with topical retinoic acid.

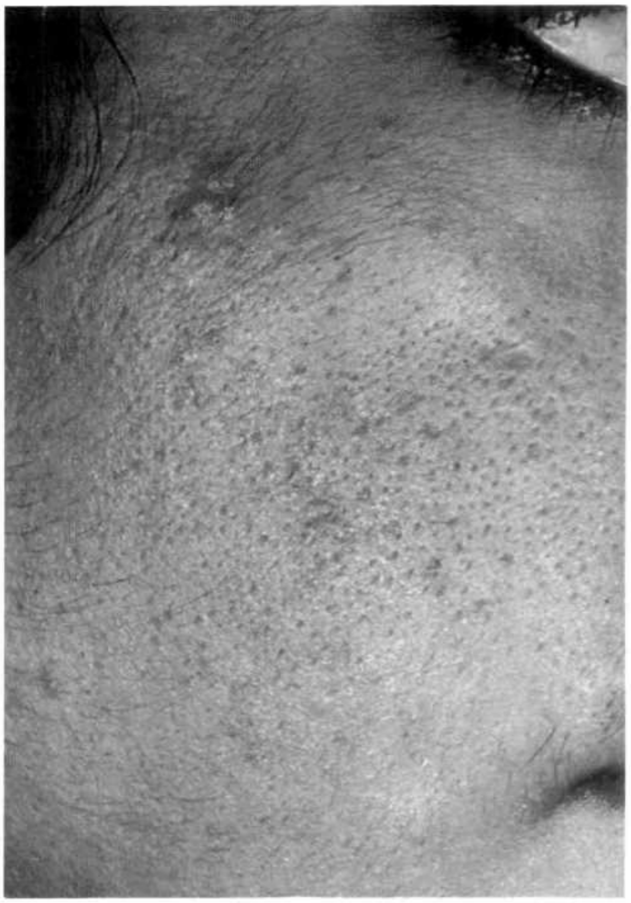

Figure 2. A marked improvement in fine acne scarring can be seen following 3 months of daily application of topical retinoic acid.

Tretinoin 0·05% has been advocated in the treatment of keloid scars[2] but there have been no reports of its use and efficacy for less aggressive scarring associated with acne vulgaris. Daily application of tretinoin 0·05% cream has been shown to have a beneficial effect on the cutaneous manifestations of photoageing through changes in both the epidermis and dermis.[3–6] Within the epidermis there is hyperplasia with a concomitant increase in the mean epidermal thickness.[7] Dermal effects reported in human skin include an increase in papillary dermal collagen and elasticity leading to improvement of dermal architecture and skin firmness.[8] In the skin of hairless mice increased numbers of hyperactive fibroblasts with concomitant formation of new collagen bundles have been found.[9] Recent studies have highlighted significant increases in elasticity after 4 months of daily application, the effect being attributed to a noticeable realignment of dermal collagen bundles.[8]

Unfortunately our patient did not consent to a skin biopsy but it would be of interest to compare the pre- and post-treatment histology to identify the changes that produced the obvious clinical improvement.

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References

1 Kligman AM, Fulton JE, Plewig G. Topical vitamin A acid in acne vulgaris. *Arch Dermatol* 1969; 99: 469–76
2 Janssen de Limpens AMP. The local treatment of hypertrophic scars and keloids with topical retinoic acid *Br J Dermatol* 1980; 103: 319–23.
3 Kligman AM, Grove GL, Hirose R, Leyden JJ. Topical tretinoin for photoaged skin. *J. Am Acad Dermatol* 1986, 15: 836–59.
4 Weiss JS, Ellis CN, Headington JT *et al.* Topical tretinoin improves photoaged skin· a double-blind, vehicle-controlled study *JAMA* 1988; 259: 527–32.
5 Leyden JJ, Grove GL, Grove MJ *et al.* Treatment of photodamaged skin with topical tretinoin. *J Am Acad Dermatol* 1989; 21: 638–440.
6 Elias PM, Williams ML. Retinoid effects on epidermal differentiation: In· *Retinoids· New Trends in Research and Therapy* (Saurat JH, ed). Basel, Switzerland: S. Karger 1985; 138–58.
7 Lever L, Kumar P, Marks R. Topical retinoic acid for the treatment of solar damage. *Br J Dermatol* 1990; 122: 91–8.
8 Berardesca E, Gabba P, Farinelli N *et al In vivo* tretinoin-induced changes in skin mechanical properties. *Br J Dermatol* 1990; 122: 525–30.
9 Kligman L. Effects of all *trans*-retinoic acid on the dermis of hairless mice *J Am Acad Dermatol* 1986; 15: 779–85.

ADONIS 000709639100173Z

Lupus erythematosus and hereditary angio-oedema

Sir, We were interested to read the report by Dr Duhra and colleages[1] regarding the use of danazol to treat a patient with discoid lupus erythematosus (LE) and hereditary angio-oedema. Although idiopathic LE was a feature in only one of 157 patients with hereditary angio-oedema reported by Brickman *et al*,[2] several previous reports suggested that this is probably a real rather than a chance association of two diseases.[3–5] We report a further case showing this association, but in whom danazol was used with limited success.

Our patient was a 26-year-old woman who had a history of angio-oedema of the extremities from the age of 3 years. She also suffered episodic abdominal pain throughout childhood, but the diagnosis of C1 esterase deficiency was not made until she was aged 21 years. About 2 years before this, she had developed marked annular lesions of discoid LE in light-exposed areas and was classified as having disseminated discoid LE. She has also had a typical 'butterfly' facial rash, occasional patchy alopecia, vague arthralgia, positive antinuclear antibody test (< 1/50, speckled pattern) and positive anti-SSA, and had two complicated pregnancies (toxaemia at age 20 years, post-partum splenic infarct at age 24 years); however, she has never fulfilled the formal criteria for systemic LE.

The diagnosis of hereditary angio-oedema was confirmed by a C1 esterase inhibitor level of 0·04 g/l in the patient, and 0·07 g/l in her 3-year-old son who was asymptomatic (normal 0·18–0·54 g/l). She also had a low C4 level (0·06 g/l, normal 0·14–0·42 g/l), C1q and C2 levels about 50% of normal, but a normal C3 level. These results suggest that she has true hereditary angio-oedema rather than the acquired form associated with the presence of anti-idiotypic antibody[6] or monoclonal protein.

Since 1983 she has been treated with systemic agents including mepacrine, chloroquine, prednisolone, tranexamic acid and danazol. Doses of danazol above 200 mg once daily caused nausea, malaise, flushing, and menstrual irregularity. At the lower dose of 200 mg daily, which was tolerated without undue side-effects, there was considerable symptomatic improvement in the angio-oedema, and improved immunological tests (C1 esterase inhibitor level 0·11 g/l and C4 level 0 13 g/l after 1 month), but she still required mepacrine 100 mg t.d.s. to control her discoid LE whilst taking this dose of danazol. We conclude that the response of LE to danazol does not necessarily parallel that of angio-oedema in patients with both these disorders, and danazol may not always be as useful as the report by Duhra and colleagues[1] would suggest.

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References

1 Duhra P, Holmes J, Porter DI. Discoid lupus erythematosus associated with hereditary angioneurotic oedema. *Br J Dermatol* 1990; 123: 241–4.
2 Brickman CM, Tsokos GC, Balow JE *et al.* Immunoregulatory disorders associated with hereditary angioedema. *J Allergy Clin Immunol* 1986, 77: 749–57.
3 Kohler PF, Percy, J, Campion WM, Smyth CJ. Hereditary angioedema and 'familial' lupus erythematosus in identical twin boys. *Am J Med* 1974; 56: 406–11.
4 Donaldson VH, Hess EV, McAdams AJ. Lupus-erythematosus-like disease in three unrelated women with hereditary angioneurotic edema *Ann Intern Med* 1977; 86: 312–13.
5 Suzuki Y, Nihei H, Mimura N, Hara M A case of hereditary angioneurotic edema associated with systemic lupus erythematosus. *Jpn J Med* 1986; 25: 281–7.

6 Melamed J, Alper CA, Cicardi M, Rosen FS. The metabolism of C1 inhibitor and C1q in patients with acquired C1-inhibitor deficiency. *J Allergy Clin Immunol* 1986; 77: 322–6.

ADONIS 000709639100174E

Premenstrual exacerbation of nickel allergy

SIR, Subjective premenstrual exacerbation of contact allergic dermatitis has been reported.[1] We used objective measurements of response to a range of concentrations of allergen, at different phases of the menstrual cycle, in patients with allergic contact dermatitis to see whether objective changes in the dose–response characteristics occurred.

Eight women, known to be allergic to nickel, who had regular menstrual cycles and were not on an oral contraceptive pill, were studied. Patch testing was performed 10 days after and 1 week before the first day of the menstrual cycle, the order of testing being varied between patients in case repeat application influenced nickel sensitivity. Two of the eight patients complained of a premenstrual exacerbation of their metal allergy and one avoided nickel at all times. None had any other premenstrual symptoms.

Six successive twofold dilutions of 5% nickel as supplied for standard patch testing (Trolab) were made in white soft paraffin. A 5-mm length of each dilution of allergen placed on a Finn chamber on Scanpor adhesive (Epitest Ltd) was applied to the upper inner arm for 48 h. At 72 h the response was measured as change in skinfold thickness using Harpenden callipers.[2]

In six of the eight patients, including one who had complained of premenstrual exacerbation of her symptoms, the responses for both clinical grade and change in skin thickness were similar at both times of the cycle, demonstrating that objective changes may not accompany subjective findings. Two patients, one who had noted premenstrual exacerbation and one who avoided nickel completely and was thus unable to identify any variation in symptoms, had much greater responses premenstrually than mid-cycle to each dose of nickel and a lower threshold for response (Fig. 1). Thus objective measurements confirmed that premenstrual exacerbation of contact allergy occurs in some patients. There was no effect of order of application of the patches.

In all patients the 5% patch-test concentration produced a positive response at both times it was tested. In practice, patch testing could fail to detect significant contact allergy in some women if performed at the middle of the menstrual cycle,[1] and patients with a positive history of contact allergic dermatitis but negative patch tests should be tested premenstrually.

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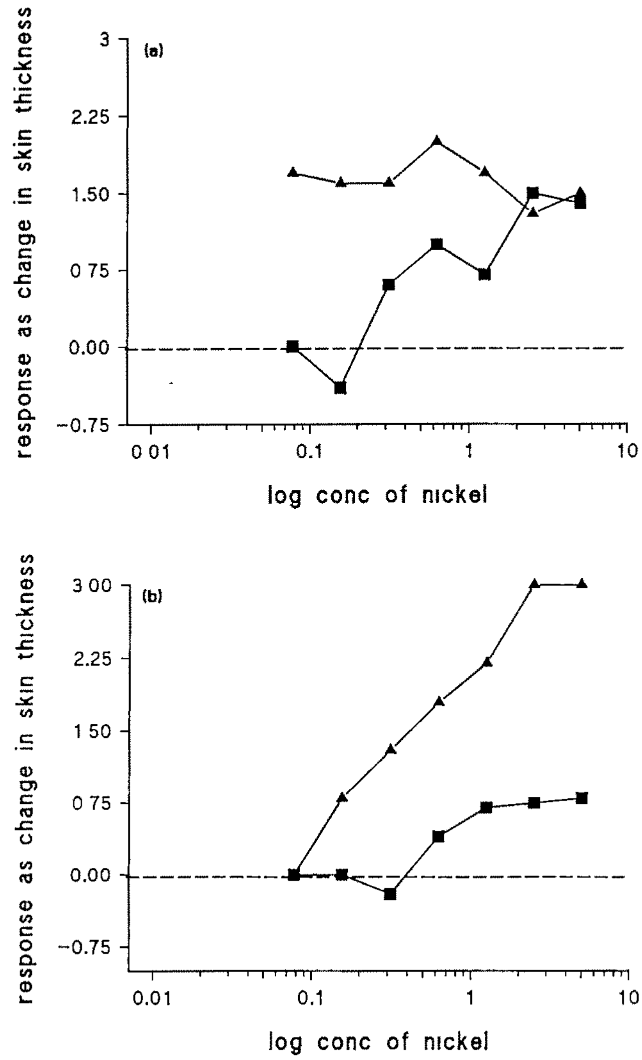

Figure 1. (a) and (b) Response measured as change in skinfold thickness in mm against log of the concentration of nickel applied for the two patients who showed premenstrual exacerbation of response ■, mid-cycle; ▲, premenstrual.

References

1 Alexander S. Patch testing and menstruation *Lancet* 1989; ii: 751.
2 McLelland J, Shuster S. Contact dermatitis with negative patch tests; the additive effect of allergens in combination. *Br J Dermatol* 1990; 122: 623–30.

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British Journal of Dermatology (1991) 125, 84–86.

Book Reviews

Dermatology (Part of the 'Brain Scan' MCQ series). ROGER ALLEN (1990). Berlin, Heidelberg: Springer-Verlag. Pp. 102. ISBN 3-540-19607-2. Price £9.95.
Self-Assessment in Dermatology. K.M.MOKBEL (1990). Wrightson Biomedical Publishing Ltd. Pp. 73. ISBN 1-871816-09-2. Price £6.50.

The true:false question format has become a widely used method of assessment in both undergraduate and postgraduate examinations. Personally, it seems that I have spent my entire medical career either answering or setting them! Drs Allen and Mokbel have produced two similar volumes of true:false questions in Dermatology. Each provides approximately 200 questions and answers with explanations. Dr Allen's style has been to divide these 200 questions into 10 practice examination papers whilst Dr Mokbel has favoured a continuous format.

I welcome the attempt in both publications to offer some advice in exam technique but feel that a word of caution should accompany the section in Dr Mokbel's book entitled 'some MCQ terms translated'. This section offers interpretations of words used in true:false questions, e.g. 'usually = more than 50%, rarely = less than 5%.' Although a useful guide the problem is that different examiners will have different interpretations of these terms. Putting aside the question of exams, both of these volumes rightly emphasize the use of such collections of questions for learning and self-assessment.

I disagree with some answers in each book—but disagreement over subtle differences in meaning is so common when groups of examiners are setting true:false questions that I prefer to dwell more on occasional flashes of humour such as when Dr Allen asks if scabies is most common in Norway.

I would recommend both volumes to students and setters of true:false questions. Those setting examinations, however, will obviously have to subtly alter the questions if a sudden glut of very high examination marks is to be avoided!

As a graduate of one of the two medical schools represented by these collections of questions I have one final nagging question. How many did I get right first time around?

R.E.A.WILLIAMS

Handbook of Pediatric Dermatology. P.A.KRUSINSKI AND F.P.FLOWERS (1990). Price £30.00.

This handbook of paediatric dermatology is written by two American dermatologists for 'clinicians who provide care for children with dermatological problems' and is intended for use by primary care physicians rather than by specialists in dermatology.

There are 18 chapters. The first deals with basic concepts and covers history taking, physical examination and diagnostic technques. Therapeutic guide-lines, with particular reference to children, are covered in Chapter two. The third is devoted to diagnostic algorithms by regional distribution. Eleven of the remaining chapters deal with diseases by the type of skin lesion: eczematous, papulosquamous, vesiculobullous and so on. There are advantages and disadvantages to this approach. It is helpful in the differential diagnosis if similar conditions can be considered together: scabies for example is covered with the eczematous conditions. However, it can also lead to conditions with a similar aetiology and treatment being covered in different chapters; viral diseases are covered in several chapters—herpes simplex (labialis and genitalis) is in the vesiculobullous section although neonatal herpes simplex is included with the pustular lesions. The three remaining chapters cover the connective tissue disorders, the photodermatoses and the life threatening dermatoses which affect children.

Although each chapter covers diseases which share clinical features, no indication is given as to their relative frequency or importance. Thus, within the vesiculo-bullous diseases benign chronic bullous disease of childhood is considered first despite its rarity, whereas herpes simplex, a common problem in children, is only considered much later. A guide to which diseases are the most common and those which, although rare, are serious would be of particular value to the non-specialist. Within a chapter, each condition is dealt with in a similar way: introduction, physical examination, laboratory investigations, therapy and discussion. Laboratory findings include both diagnostic tests such as mycological examination and also cover the histological appearances. Although a description of the histological features is useful for completeness it should be stressed that most of the conditions discussed do not justify a skin biopsy in a child.

A handbook can fall into one of two main types. It can either highlight those conditions most commonly seen in clinical practice or aim to be more comprehensive. The first approach is the more common and, in dermatology, is often centred around clinical photographs. The second is usually presented as lecture notes or a shortened textbook. This book chooses the second approach. Handbooks of paediatric dermatology have a particular problem: in an effort to be comprehensive the text becomes more a textbook of general dermatology than one focused on children. This book does not always avoid this problem. For example a helpful diagnostic sign of scabies in infants is the involvement of the soles, often with bullous lesions. This is not stressed in the text although it comments on the nodular penile lesions in males, a much more helpful feature in adults.

The book has 77 colour plates. These are contained in a separate section at the beginning of the book. Unfortunately many of the plates are too small to demonstrate adequately the clinical features illustrated. In addition, throughout the text, there are a number of black-and-white plates; many of these are too poor to be of value. There are also a number of tables of the less common disorders. These are useful and well presented and perhaps more of the text could have been handled in this way allowing more space for the more common disorders.

On balance this book provides a fairly comprehensive review of paediatric dermatology for the non-specialist. It is spoilt by the poor illustrations which is unfortunate as the format of the book presupposes that the clinician can recognize the skin lesions to be eczematous, papulosquamous, an exanthem and so on. Primary care physicians might prefer an atlas or well-illustrated handbook, which can be supplemented for the less common conditions by the larger textbooks of dermatology.

R.Lever

Immunologic Diseases of the Skin. Edited by Robert E.Jordan (1991). East Norwalk, Connecticut: Appleton and Lange. Pp. 646. ISBN 0-8385-4037-6. Price: $US 125.

This book provides a digestible approach to a better understanding of those skin disorders thought to be primarily a result of defective immune function. It is aimed principally at the clinician wishing to be updated on the bewildering advances which have occurred in the past decade or so. However, the large volume of work reviewed and referred to, also makes this an excellent reference for investigative dermatologists. The book's main strength is its detailed evaluation of the immunological abnormalities and immunopathological findings detectable in a wide variety of different skin disorders. Eighty-one contributors (five from outside the U.S.A.) have written a total of 52 chapters, laid out in two parts. The first section entitled 'Basic Immunology' is a clear review of those immune mechanisms relevant to human skin, together with a more in-depth look specifically at the roles of lymphocytes, mast cells and eosinophils. The remaining part, entitled 'Immunologic Cutaneous Disease' addresses a wide variety of skin disorders which are grouped under headings such as Immuno-Deficiency Disorders, Cutaneous Allergy, Auto-immune Diseases—Bullous Disease Group, Auto-immune Diseases—Rheumatic Disease Group, Immune Complex Diseases, etc. Each disorder is dealt with in terms of its general background, clinical features and treatment aspects, but where this book scores over others is in its approach to underlying immunology and pathomechanisms. In many instances the contributors have reviewed in some detail the available immuno-pathological data on a disorder and attempted to synthesize this into some form of scheme for pathogenesis. This is an invaluable approach for the reader with an interest in pathomechanisms of cutaneous disease, but without the time to update on the investigative literature him/herself. The reference sections for each chapter are extensive and up to date. The clinical illustrations are adequate and the pathological illustrations are good. The more in depth approach to the pathogenesis of skin disease offered in this book will provide valuable reading to both the interested clinician and the active investigator.

J.Ashworth

Medical Statistics on Microcomputers. R.A.Brown, J.Swanson Beck (1990). London: British Medical Journal. Pp. 103. ISBN 0-7279-0290-3. Price £8.95.

At a recent statistics course I attended, great emphasis was placed on the graphical exploration of data and the use of confidence intervals rather than significance testing in the comparison of experimental groups. The use of statistics programs on microcomputers is probably the easiest way to achieve both of these aims and this publication, bringing together a series of papers originally published in the *Journal of Clinical Pathology*, is the ideal companion.

Medical Statistics on Microcomputers has two main strengths. Firstly, it does not pretend to be a comprehensive statistical reference book but in systematically considering the commonly used statistical techniques it concentrates on clinical and investigatory situations. Suitable ways to tackle each problem are suggested but it is also swift to highlight common errors, such as the use of correlation to compare two laboratory techniques or analyses generating multiple comparisons.

The book's second strength is to illustrate the different options available to display data graphically. These are not confined to the well known scatter plots and histograms but will introduce many clinicians to the very useful 'box and whisker' and dot plots. The ease by which these different plots can be generated on a microcomputer makes it easy for the investigator to develop a proper 'feel' for his own data.

Throughout the chapters examples of how to use two popular statistics programs, namely STATGRAPHICS and MINITAB, are given. I am familiar only with MINITAB but the information provided for this package is helpful especially as I personally find the MINITAB manual rather unfriendly. One slightly irritating feature is that in a few cases information is provided for only one of the two statistical packages and it would be interesting in those cases to see how the alternative package addressed the same problem.

I would recommend this publication to all who analyse data on a microcomputer. It is a worthy addition to the *British Medical Journal* stable of practical statistics guides.

R.E.A.Williams

Pathology of the Skin. Edited by E.R.Farmer and A.F.Hood (1990). East Norwalk, Connecticut: Appleton and Lange. Pp. 987. Illustrations: 1004. ISBN 0-8385-7724-5. Price £90.00.

Pathology of the Skin, edited by E.R.Farmer and A.F.Hood, has the appearance of a sober, slightly old-fashioned text, with its dull brown binding and it certainly is a serious attempt to 'provide an authoritative and scholarly survey of dermatopathology'. Whether it is likely to be 'a practical format useful to dermatopathologists, pathologists and dermatologists' is arguable but whilst I personally found much to appreciate, it is idiosyncratic and variable in its presentation and quality.

The editors have called upon 64 collaborators, all but four of whom are American, to contribute in their special areas. The editors admit that they had more difficulty in achieving uniformity of style with some authors than others. The most striking overall feature of the book, which is not necessarily a defect, is the number of subjects which are covered in more than one chapter. The views expressed in different chapters are often contrasting which gives, perhaps unintentionally, a very balanced view. Examples of these repetitions include eczema, seborrhoeic dermatitis, angiolymphoid hyperplasia and Kimura's disease, intraepidermal epithelioma ('Jadassohn'), Bowen's disease, keratoacanthoma and benign melanocytic lesions.

By far the largest chapter written in the style of a monograph is that on dysplastic naevi and melanoma by the Philadelphia group. Whilst this is little short of brilliant it remains very much the expression of one group with few concessions to any other work. The style of this dominant chapter is persuasive but becomes somewhat portentous with sections on humanistic and moral considerations which seem oddly sited in a pathology text. Convincing though much of this chapter is, it is much less so in describing acral lentiginous melanoma and displays further dogmatism in the assessment of regression.

The chapter on melanoma contrasts with an unexpectedly short section on cutaneous lymphomas and with the numerous short chapters of 3–4 pages each, covering their topics rather briefly. However some of these shorter chapters such as those on neural and vascular tumours and hair and mucosal problems are lucid and very helpful, but I do take exception to the description in the chapter on inflammatory reactions of the sweat unit, of hidradenitis suppurativa as an inflammatory disease of apocrine sweat glands. Despite its name it is better classified as a follicular occlusion problem.

Many of the other 61 chapters provide clear explanations of their subject but throughout the book, the black-and-white photography often does not illustrate the intended points. Many photographs are simply not clear, are at too low magnification or are too small and not well centred or illuminated. In this aspect the contrast with McKee's book of the same title is extreme.

Some of the chapters in Section 1 on General Considerations seem superfluous for the intended audience. This comment applies particularly to the chapters on specimen preparation, histochemistry and methodology of electron microscopy. Anyone who wanted guidance in these areas would use more specialized texts or personal tuition. Deletion of these sections together with some of the topic repetitions would considerably shorten this large book. The book has good references up to 1988 and most importantly includes crucial papers from the older literature.

Making a choice between this book and 'Lever' or 'McKee' is difficult. It certainly is in places more detailed and penetrating than both. 'Lever' has the uniformity of style and cohesion by having the same authors for the whole book. This is both an advantage and disadvantage because authors of specialist sections in 'Farmer and Hood' clearly show greater authority. The illustrations are less satisfactory than 'Lever' and both these books are at a conspicuous disadvantage to 'McKee' which has colour photographs throughout and good clinical illustrations Nevertheless, the text, if you can tolerate the unusual arrangement of chapters and the repetition, is generally more detailed than 'McKee' and usually more so than 'Lever'.

Whether the authors have achieved their aim of blending a disease orientated approach with the more modern pattern-recognition approach is doubtful. I see little evidence of the latter and would not regard this as a bench-top diagnostic manual in the way that Ackerman's text on inflammatory diseases of skin so obviously succeeds. The index, as far as I have used it, works well which is in marked contrast to that of 'McKee'.

Despite these reservations *Pathology of the Skin* is a book with character which, with tighter editorial control, should evolve into a text worthy to compete for the dominant position held for so long by 'Lever'. It already has many sections which can be read with benefit and pleasure. The price at £90.00 seems very competitive.

M.Cook

Book Received

Thesis. Evaluation de L'etat Fonctionnel de la Peau par des Methodes Non Invasives. D.Van Neste. Skinterface sprl, 1991.

New editions

Syndromes of the Head and Neck, 3rd edn. R.J. Gorlin, M.M. Cohen Jr and L.S. Levin (1990). Oxford Monographs on Medical Genetics No. 19. Oxford University Press. Pp. 977. ISBN 0-19-504518-1. Price £150.

This is the third edition of a well recognized classic which should be available in every dermatological library.

Fungal Infection in the Compromised Patient, 2nd edn. Edited by D.W. Warnock and M.D. Richardson (1991). Chichester: John Wiley & Sons Limited. Pp. 371. ISBN 0-471928410. Price £47.50.

This is the second edition of an extremely useful guide to fungal problems in immunosuppressed patients. It has been substantially updated in the light of recent experience with HIV patients. The book is recommended.

British Journal of Dermatology (1991) 125, 87–88.

News and Notices

Second Dermatopathology Self-Assessment Course, 19–21 September 1991, Rome

The course will provide an in-depth review of two difficult topics in dermatopathology: cutaneous vascular pathology (A.B.Ackerman) and skin soft tissue tumours (S.W.Weiss).

The programme will also consist of a series of lectures and direct examination of slides. Participants are also invited to present their own cases which will be published in the abstract book. The course fee is US$150 for participants in all the course and US$70 for those desiring to attend only the lectures.

For further information contact: Dr Gido Massi, Department of Pathology, Catholic University, Largo F. Vito 1, 00168 Rome, Italy. Telephone: (6) 3303481/33054270/5759733.

French Society for Dermatological Research 27–28 September 1991, Marseille

The annual congress of the French Society for Dermatological Research will be held in Marseille on 27–28 September 1991. For further information contact: Dr P.Berbis, Clinique Dermatologique, Hôtel Dieu, 13002 Marseille, France. Telephone: 33-91-96-49-66.

12th Annual Conference of the Australian Dermatopathology Society, 27–29 September 1991, Melbourne, Australia

The 12th Annual Conference of the Australian Dermatopathology Society will be held at St Vincent's Hospital, Melbourne on 27–29 September 1991. The guest speaker will be Professor A.B.Ackerman, New York University Medical School. The Conference on Interface Dermatopathology will highlight clinical and laboratory contributions to diagnosis and will include five-minute presentations and poster displays. For further information, please contact: Mrs Ann Dancer, Victorian Medical Postgraduate foundation Inc (VMPF), P.O. Box 27, Parkville, Victoria 3052, Australia. Telephone: 010-61-3-347-9633.

Dermatology '91: Therapeutic Update 27–29 September 1991, Vancouver

Dermatology '91: Therapeutic Update, the ongoing dermatology drug symposium series, will take place on 27–29 September 1991 at the New World Harbourside Hotel, Vancouver, British Columbia, Canada. For registration and further information, contact: Symposium Secretariat, Events by Design, 601-325 Howe Street, Vancouver, BC, Canada V6C 1Z7. Telephone: 604-669-7175.

Symposium on Epidermolysis Bullosa: Molecular Biology and Pathology of the Basement Membrane Zone, 4–5 October 1991, Philadelphia, U.S.A.

A Symposium on Epidermolysis Bullosa, Molcular Biology and Pathology of the Basement Membrane Zone will be held on 4–5 October 1991, sponsored by the Jefferson Medical College, National Institutes of Health, and Dystrophic Epidermolysis Bullosa Research Association of America. For further information contact: Eileen O'Shaughnessy, Department of Dermatology, Jefferson Medical College, Roopm M-46 JAH, 1020 Locust Street, Philadelphia, PA 19107, U.S.A. Telephone: 215-955-5785. Fax: 215-955-5788.

Update in Cutaneous Laser Surgery, 12–13 October 1991, Cambridge, Massachusetts

The Departments of Dermatology and Plastic Surgery of the Beth Israel Hospital, Children's Hospital and New England Deaconess Hospital, and Harvard Medical School, Boston, Massachusetts are jointly sponsoring the course 'Update in Cutaneous Laser Surgery' on 12–13 October 1991 at the Hyatt Regency Hotel in Cambridge, Massachusetts. The course will address all theoretical and practical aspects of cutaneous laser surgery. Hands-on workshops will be provided with various types of laser instruments including argon, continuous wave and pulsed dye, carbon dioxide, neodymium yag, pulsed ruby and copper vapour lasers. The course will include formal lectures, panel discussions and videos of demonstrations of laser techniques and laser safety. For further information contact: the Department of Continuing Medical Education, Harvard Medical School, 25 Shattuck Street, Boston, MA 02215, U.S.A. Telephone 617-732-1525.

Symposium on Facial Malignancy and Reconstruction, University of Minnesota, 25–26 October 1991

A symposium will be held on Facial Malignancy and Reconstruction at the Radisson Hotel Metrodome, University of Minnesota, Minneapolis, Minnesota. For further information contact: Continuing Medical Education, University of Minnesota, Radisson Hotel Metrodome, Suite 107, 615 Washington Avenue SE, Minneapolis, MN 55414, U.S.A. Telephone (612) 626-7600.

6th Annual Cosmetic Dermatologic Surgery Seminar/Dialogues in Cosmetic Dermatology, 30 October–3 November 1991, Orlando, Florida

The 6th Cosmetic Dermatologic Surgery Seminar and Dialogues in Cosmetic Dermatology will be held at Walt Disney World/Yacht and Beach Club in Orlando, Florida on 30 October–3 November 1991. For further information contact: Dr J.B.Pinski, 55 E Washington Street, Suite 3404, Chicago, Illinois 60602, U.S.A. Telephone: 312-263-4625.

International Symposium on Topical Administration of Drugs, 27–29 January 1992, Stockholm, Sweden

An International Symposium will be held on the Topical Administration of Drugs in Stockholm sponsored by the Swedish Academy of Pharmaceutical Sciences, 27–29 January 1992. Sessions will be held on fundamentals of skin permeation, formulation, pharmacokinetics, *in vitro* and *in vivo* systems, enhancer systems, clinical and dermatotoxicological aspects and visions of the future. For further information please contact: the Swedish Academy of Pharmaceutical Sciences, P.O. Box 1136, S-111 81 Stockholm, Sweden. Telephone 46-8-24-50-85.

CLINICAL AND EXPERIMENTAL ALLERGY

Formerly Clinical Allergy
JOURNAL OF THE BRITISH SOCIETY FOR ALLERGY
AND CLINICAL IMMUNOLOGY
Edited by **A.B. Kay** and **S.T. Holgate**

Clinical Allergy has been known as ***Clinical and Experimental Allergy*** since 1st January 1989 and is the official journal of the British Society for Allergy and Clinical Immunology. The success of *Clinical Allergy* resulted largely from the ability to supply practising allergists with information based on clinical science experimentation, yet at the same time the editors maintained the interests of basic scientists. This tradition has been continued, and also improved upon. Review articles are included together with editorials, letters to the editor and book reviews. Some new features that have been incorporated include *current abstracts, allergy practice forum,* and *news and commentary.* Papers on drug trials that shed light on pathogenetic mechanisms or present the results of entirely novel pharmacological agents are also included. Educational items such as the allergy practice forum are of special interest to clinical allergists world wide.

Clinical Allergy was first published in 1971, the founding editor being Professor Jack Pepys. Between 1980 and 1983 the Journal was co-edited by Dr James Kerr and Dr William Parish. Most of the 1984 issues and the first two issues of 1985 were managed entirely by Dr Parish. The current co-editors are Professor Barry Kay and Professor Stephen Holgate who took over the editorship in 1985.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be *unmounted* glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets: they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it: these should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data: they should be typewritten on separate sheets and should be given Arabic numbers.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g
kilogram(s)	kg
milligram(s) (10^{-3}g)	mg
microgram(s) (10^{-6}g)	*μ*g
nanogram(s) (10^{-9}g)	ng
picogram(s) (10^{-12}g)	pg
hours(s)	h
minute(s)	min
centimetre(s)	cm
second(s)	s
cubic millimetre(s)	mm^3
millimetre(s)	mm
millicurie(s)	mCi
milliequivalent	mmol
molar	mol l
millilitre(s)	ml
gravitational acceleration	*g*
micrometre(s)	*μ*m
per cent	%
isotopic mass number placed as	^{131}I

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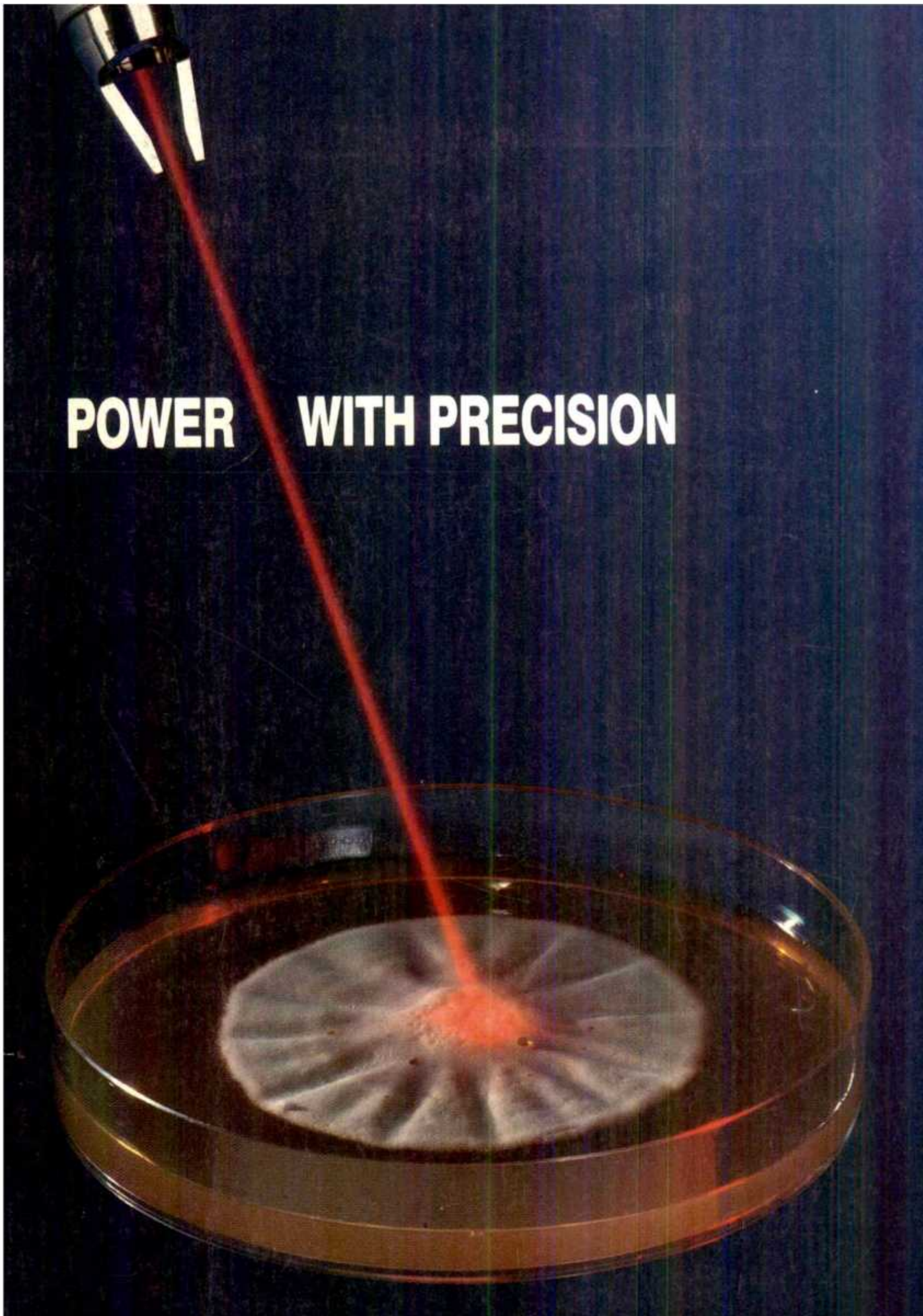

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Presentation Tablets providing 250mg of terbinafine. **Principal Action** Terbinafine is an allylamine which has a broad spectrum of antifungal activity. At low concentrations terbinafine is fungicidal against dermatophytes, moulds, certain dimorphic fungi and yeasts. **Indications** Fungal infections of the skin caused by *Trichophyton*, *Microsporum canis* and *Epidermophyton floccosum*. **Dosage and Administration** *Adults:* One 250mg tablet daily. **Contra-indication** Hypersensitivity to LAMISIL. **Precautions and Warnings** Patients with severe liver dysfunction or severely impaired renal function should receive half the normal dose. LAMISIL should not generally be used during pregnancy. Mothers should not receive LAMISIL whilst breastfeeding. **Side Effects** Side effects are mild to moderate and transient. Gastrointestinal symptoms (fullness, loss of appetite, nausea, mild abdominal pain, diarrhoea). Allergic skin reactions (rash, urticaria). **Further Information** Terbinafine rapidly diffuses through the dermis and concentrates in the lipophilic stratum corneum. Terbinafine is also secreted in sebum, thus achieving high concentrations in hair follicles, hair and sebum rich skins. There is also evidence that terbinafine is distributed into the nail plate within the first few weeks of commencing therapy. **Package Quantities and Basic NHS Cost** 14 tablets in a calendar pack £23.75. 28 tablets in a calendar pack £45.80. **Product Licence Number** PL 0101/0304. LAMISIL is a registered Trade Mark. Full prescribing information, including product data sheet, is available from: Sandoz Pharmaceuticals, A division of Sandoz Products Limited, Frimley Business Park, Frimley, Camberley, Surrey GU16 5SG.

References 1. Clayton YM. Clin Exp Dermatol, 1989, **14**, 2, 101-3. 2. Ryder NS. Antimicrob Agents Chemother, 1985; **27**, 252-6. 3. Schuster I. Xenobiotica, 1985, **15**, 529-46. 4. Schuster I, Ryder NS. J Dermatol Treat, 1990, 1, (Suppl 2), 7-9. 5. Villars V, Jones TC. J Dermatol Treat, 1990, 1, (Suppl 2), 33-8. 6. Savin R. Clin Exp Dermatol, 1989, **14**, 2, 116-19. 7. White JE, Perkins P, Evans EGV. Br J Dermatol, 1990, **123**, (Suppl 37), 30.

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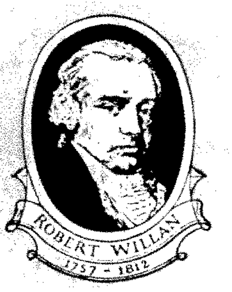

British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications

British Journal of Dermatology

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SUBSCRIPTION INFORMATION. *British Journal of Dermatology* is published monthly (2 volumes per annum) and the subscription prices for 1991 are £119.50 (U.K.), £130.00 (overseas except North America), $237.00 (U.S.A. and Canada), in all cases post free. Orders for current subscriptions and back issues should be sent to Journal Subscriptions Dept., Marston Book Services, P.O. Box 87, Oxford, U.K. Tel. 0865 791197, Telex 837515 Mardis G. Fax 0865 721205; all other business correspondence, including orders for offprints and advertising space, should be addressed to Blackwell Scientific Publications Ltd., Osney Mead, Oxford OX2 0EL (telephone no. 0865 240201, Telex 83355 Medbok G. Fax 0865 721205).

DESPATCH. The Journal is despatched within Europe by surface mail, to other continents by various forms of air-speeded delivery: to the U.S.A.* by air freight for forwarding by second-class post, to India by air freight for guaranteed local delivery, and to all other countries by Accelerated Surface Post.

* Second-class postage paid at Rahway, NJ. Post Master, send address changes to *British Journal of Dermatology*, c/o Mercury Airfreight International Inc., 2323 Randolph Avenue, Avenel, NJ 07001, U.S.A.

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This journal is printed on acid-free paper.

ABC

Printed in Great Britain at the Alden Press, Oxford

British Journal of Dermatology

Volume 125, Number 2, August 1991

Acc 24/1/95

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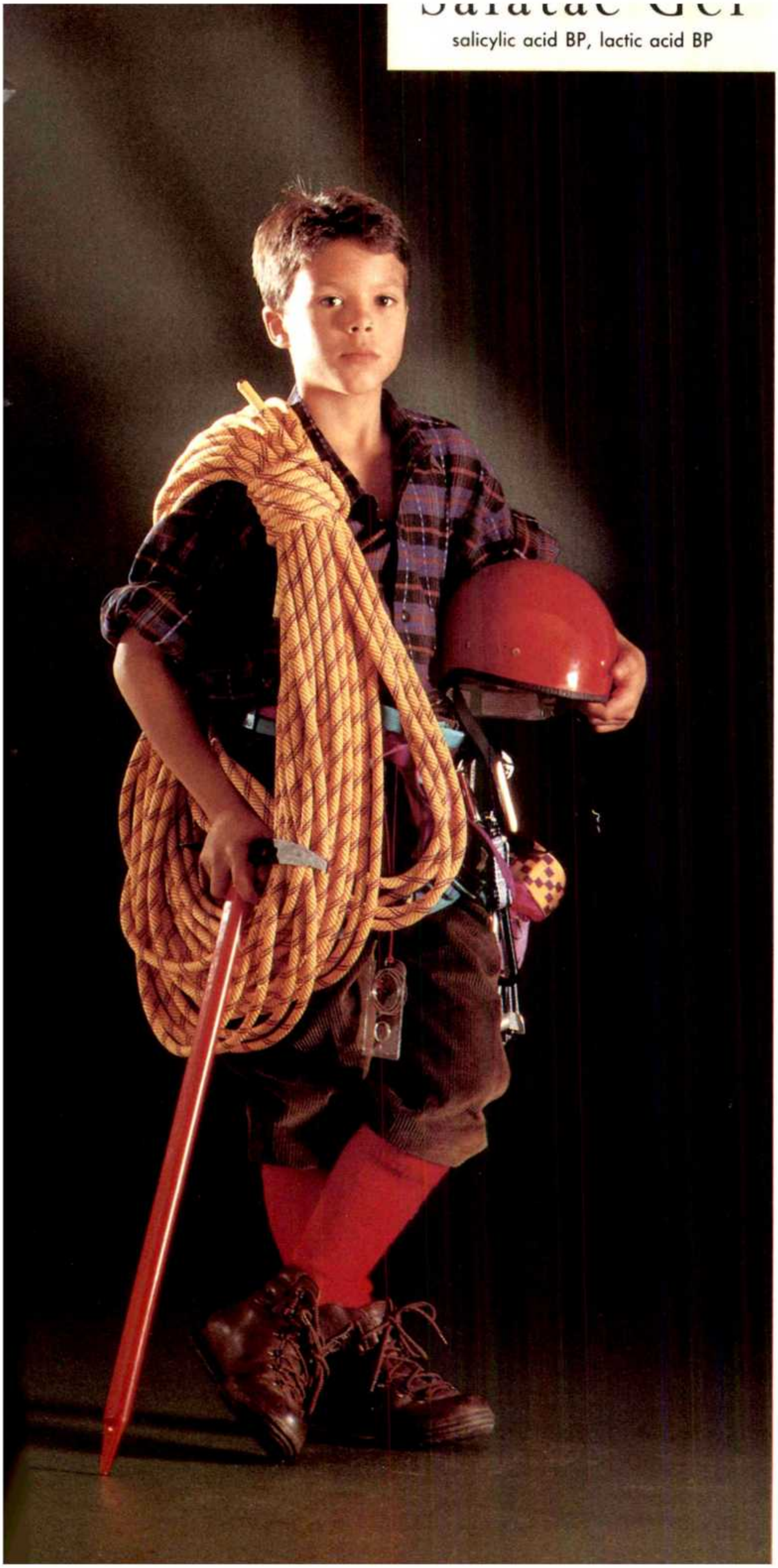

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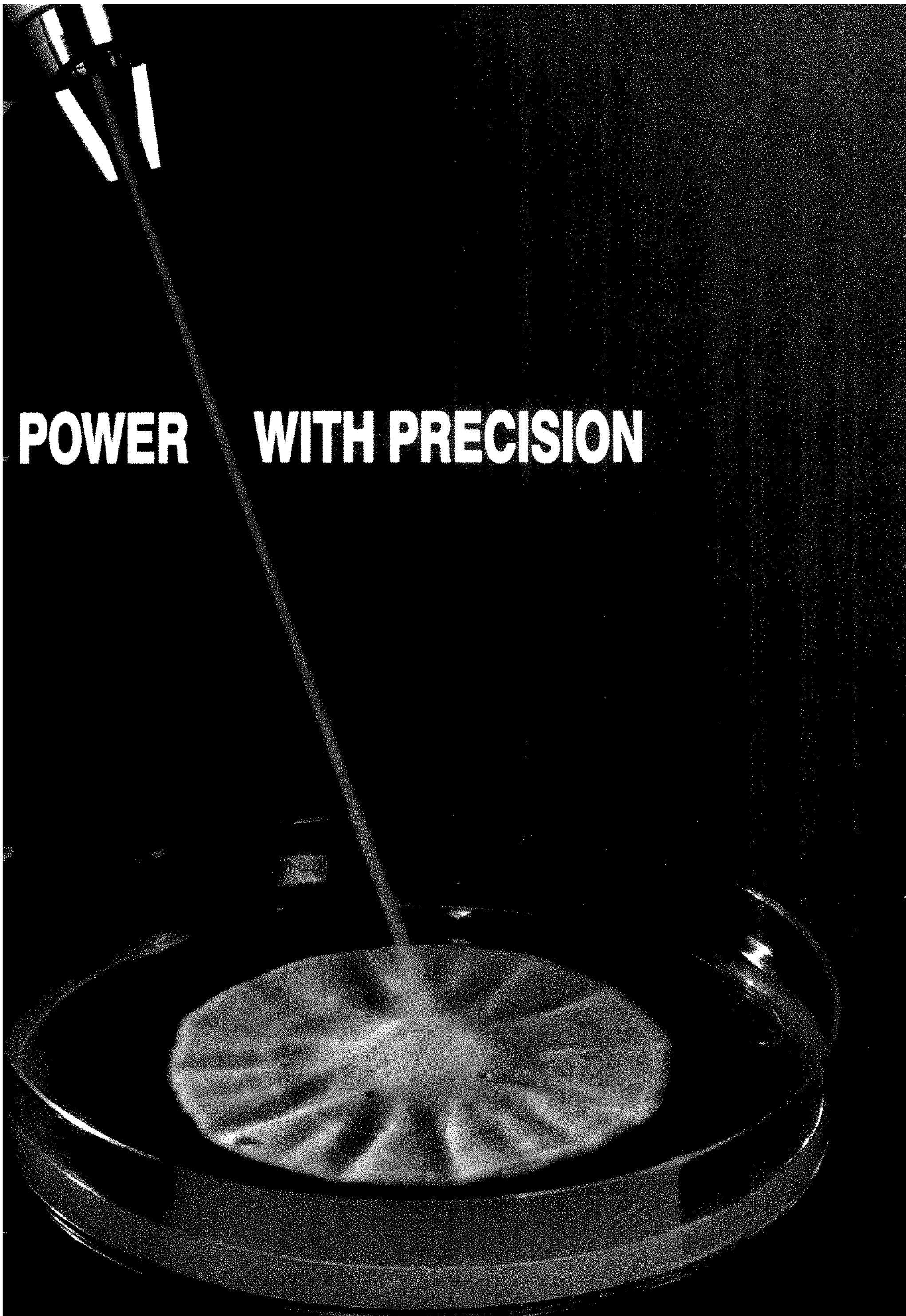

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breviated Prescribing Information
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tivity. At low concentrations terbinafine is fungicidal against
rmatophytes, moulds, certain dimorphic fungi and yeasts. **Indications**
ngal infections of the skin caused by *Trichophyton*, *Microsporum canis*
d *Epidermophyton floccosum*. **Dosage and Administration** *Adults*:
1e 250mg tablet daily. **Contra-indication** Hypersensitivity to LAMISIL.
ecautions and Warnings Patients with severe liver dysfunction or
verely impaired renal function should receive half the normal dose.
.MISIL should not generally be used during pregnancy. Mothers should
t receive LAMISIL whilst breastfeeding. **Side Effects** Side effects are
mild to moderate and transient. Gastrointestinal symptoms (fullness,
loss of appetite, nausea, mild abdominal pain, diarrhoea). Allergic
skin reactions (rash, urticaria). **Further Information** Terbinafine
rapidly diffuses through the dermis and concentrates in the
lipophilic stratum corneum. Terbinafine is also secreted in sebum, thus achieving high concentrations in hair follicles, hair and sebum rich skins. There is also evidence that terbinafine is distributed into the nail plate within the first few weeks of commencing therapy. **Package Quantities and Basic NHS Cost** 14 tablets in a calendar pack £23.75. 28 tablets in a calendar pack £45.80. **Product Licence Number** PL 0101/0304. LAMISIL is a registered Trade Mark. Full prescribing information, including product data sheet, is available from: Sandoz Pharmaceuticals, A division of Sandoz Products Limited, Frimley Business Park, Frimley, Camberley, Surrey GU16 5SG.

References 1. Clayton YM. Clin Exp Dermatol, 1989, **14**, 2, 101-3. 2. Ryder NS. Antimicrob Agents Chemother, 1985; **27**, 252-6. 3. Schuster I. Xenobiotica, 1985, **15**, 529-46. 4. Schuster I, Ryder NS. J Dermatol Treat, 1990, 1, (Suppl 2), 7-9. 5. Villars V, Jones TC. J Dermatol Treat, 1990, 1, (Suppl 2), 33-8. 6. Savin R. Clin Exp Dermatol, 1989, **14**, 2, 116-19. 7. White JE, Perkins P, Evans EGV. Br J Dermatol, 1990, **123**, (Suppl 37), 30.

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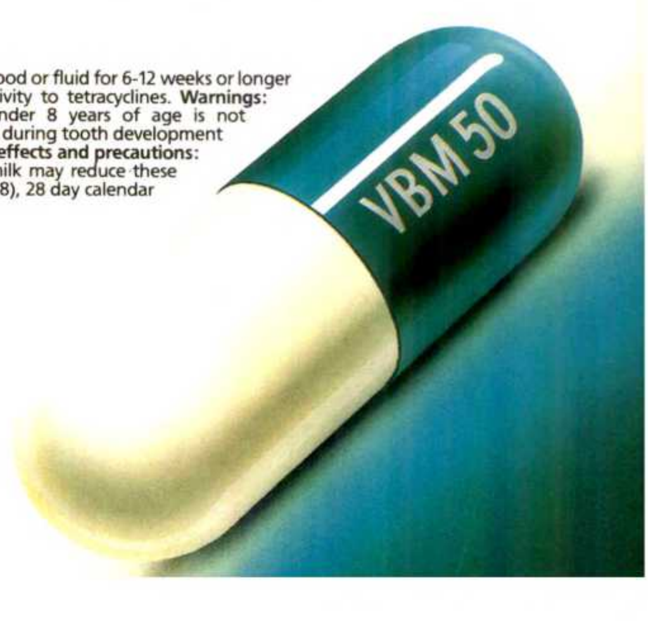
Full information available on request.

Reference
1. Harrison, P.V., (1988) Clin. Exp. Dermatol 13, 242-244



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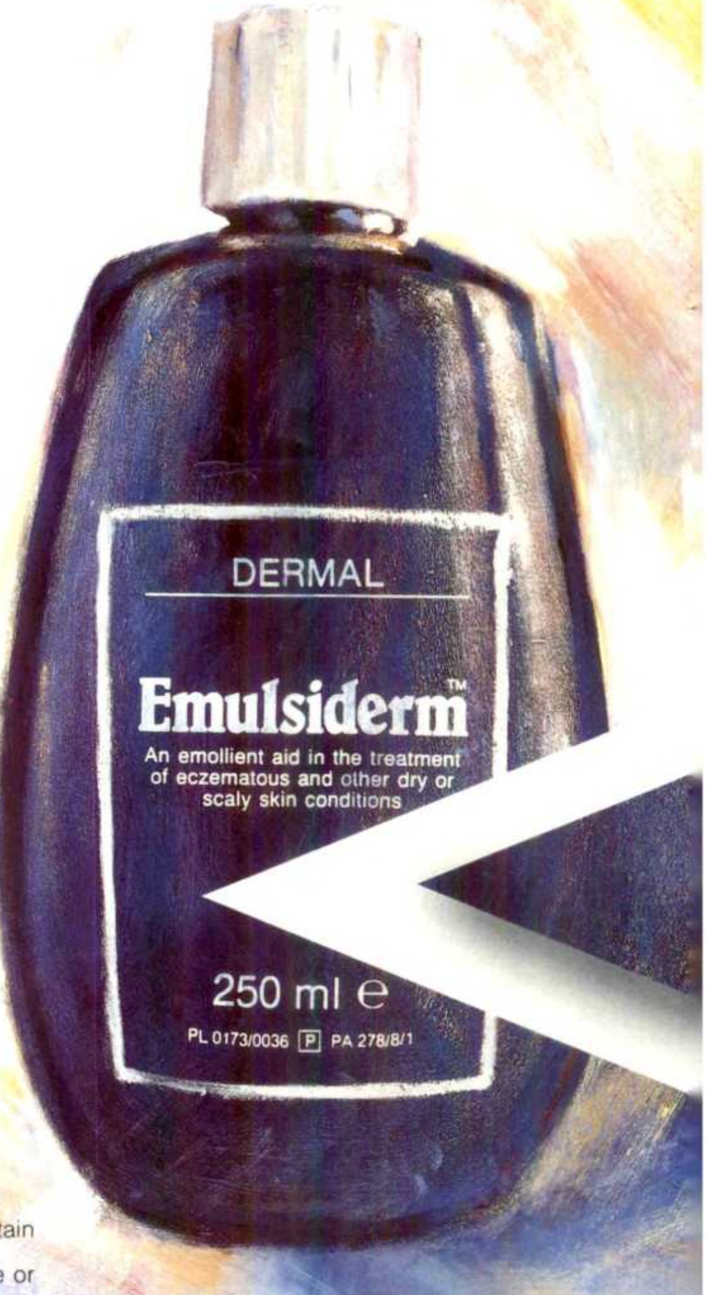
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British Journal of Dermatology (1991) **125**, 89–93.

ADONIS 0007096391001766

REVIEW

The treatment of human ectoparasite infection

D. A. BURNS

Department of Dermatology, Leicester Royal Infirmary, Leicester LE1 5WW, U.K.

Accepted for publication 1 May 1991

Head-louse infection[1–3]

The acetylcholinesterase-inhibiting insecticides malathion and carbaryl are currently the mainstay of treatment for head-louse infection in the United Kingdom. Malathion was introduced for head-louse treatment in 1971,[4] following evidence of the emergence of resistance to the organochlorine insecticides. The effectiveness of carbaryl as a pediculicide in clinical practice was demonstrated a decade later.[5] Both malathion and carbaryl are efficient pediculicides, and good ovicides,[4–8] although the ovicidal activity is not complete.

Malathion is adsorbed on to keratin, a process which takes approximately 6 h, and confers residual protective effect against re-infection for about 6 weeks.[4,6] Although carbaryl was originally considered to provide a similar residual protective effect[5] this is not now thought to be the case.

There is evidence that the effectiveness of proprietary preparations containing carbaryl is determined to some extent by components of the bases utilized in these preparations, and that they vary in their pediculicidal and ovicidal activity.[9] The variation in insecticidal activity is particularly noticeable when short application times are employed.

Malathion and carbaryl preparations should remain on the scalp for 12 h before being washed off. Both insecticides are degraded by heat, and a hot-air drier should not be used to dry the hair. Because of the incomplete ovicidal activity treatment should be repeated after an interval of 7–10 days. The majority of Health Districts in the U.K. now alternate the use of malathion and carbaryl every 3 years in an attempt to avoid development of resistance to these insecticides and to prolong their useful lives. Lists providing details of insecticide use in U.K. Health Districts have been published in *The Pharmaceutical Journal* to encourage retail pharmacies to follow their local Health District policy when dispensing over-the-counter.[10]

Also effective as pediculicides and ovicides are pyrethrins and synthetic pyrethroids whose development and use has recently been comprehensively reviewed by Taplin and Meinking.[11] Pyrethrins are naturally occurring insecticides extracted from plants of the genus *Crysanthemum*, and in the United States they are marketed as a number of over-the-counter preparations for the treatment of head lice and crab lice. They are combined with piperonyl butoxide, a powerful synergist for natural pyrethrins. Synthetic pyrethroids, developed from natural pyrethrins, have greater insecticidal activity. They also have the advantages of being photostable and biodegradable, and having low mammalian toxicity. The synthetic pyrethroid permethrin has been extensively evaluated in the treatment of head-louse infection.[12–16] At present in the U.K. there are three over-the-counter synthetic pyrethroid preparations marketed for the treatment of head lice: a 1% permethrin cream rinse (Lyclear), which is a 10-minute treatment applied after shampooing the hair, and two preparations containing phenothrin, a shampoo (Full Marks Shampoo) and an alcohol-base lotion (Full Marks Lotion). A 1% permethrin cream rinse is marketed in the U.S.A. with the trade name Nix.

The efficacy of oral cotrimoxazole in eradicating head lice was reported by Shashindran *et al.*,[17] and subsequently by Campos *et al.*[18] This effect is probably related to ingestion of the antibiotic by feeding lice, and its subsequent effect on their symbiotic bacteria.[19] These bacteria which are present in a mid-gut organelle known as a mycetome are essential for the synthesis of B vitamins without which the lice cannot survive. Topical 10% crotamiton lotion has also been reported by Karacic and Yawalkar as being an effective treatment for head lice.[20]

Empty egg cases persist on the hair shafts for some time, until they are gradually worn away by repeated washing. They are otherwise difficult to dislodge, and nit-combs are of limited value. De Felice *et al.*[21] recently reported the effectiveness of a cream rinse containing 8% formic acid as a nit-removal system.

Clothing lice

In clothing louse infection it is the clothing, and not the patient, which requires treatment. High temperature laundering of undergarments, and dry-cleaning of outer clothing, will kill lice and eggs. Tumble-drying is also a most effective way of killing lice and eggs in clothing.[22]

In communities where clothing louse infection is an endemic problem, insecticides are employed to treat clothing. The emergence of lice resistant to DDT and lindane necessitated the introduction of malathion dusting powder for louse control,[23,24] and recently permethrin-treated clothing has been shown to be toxic to clothing lice.[25]

Crab lice

Preparations containing malathion, carbaryl, gamma benzene hexachloride (lindane), synergized pyrethrins and synthetic pyrethroids may all be used for the therapy of crab-louse infection.[26–28] Alcohol-base preparations may be irritant when applied to the scrotum, and aqueous preparations such as Quellada lotion (lindane) or Derbac-M liquid (malathion) are preferable. In view of the frequent involvement of other hairy areas of the body, in addition to the pubic region, it is advisable to treat the whole of the trunk and limbs. Treatment should be repeated after an interval of 7–10 days. All sexual contacts should also be treated when possible.

Eyelash infection[29, 30]

Although mechanical removal of lice and eggs with fine forceps[31] or epilation of the lashes with their attached eggs are obvious methods for dealing with eyelash infection, these procedures are uncomfortable, are not usually feasible in small children, and are not recommended. Several other methods of treating crab lice on the eyelashes have been suggested, including cryotherapy,[32] fluorescein in concentrations of 10–20%,[33] petrolatum,[27,34] and physostigmine eye ointment.[29,35,36] A drawback of physostigmine is that it paralyses accommodation and causes blurred vision if introduced into the eye. Modern insecticide preparations may be used to treat crab lice on the eyelashes, but alcohol-base preparations are irritant if inadvertently introduced into the eye, and aqueous preparations such as malathion 0·5% liquid (Derbac-M liquid) are preferable.[30]

Scabies

Many remedies for scabies have been suggested in the past. Sulphur preparations have been used for centuries, and sulphur ointment is still employed by dermatologists in some parts of the world as an effective and inexpensive treatment. If used excessively or in high concentration sulphur causes irritation, but with correct usage 10% sulphur in yellow soft paraffin is safe and effective. At a concentration of 2·5% it may be used for the treatment of scabies in infants.[37]

There are several currently available scabicides, and choice of preparation is usually determined by the particular preference of the dermatologist, the degree of secondary eczematization, and the age of the patient. Although for many years a hot bath and vigorous scrubbing of the sites of burrows was considered an essential part of the scabies treatment regime, Mellanby *et al.*[38] demonstrated that this is not necessary.

Benzyl benzoate

Although benzyl benzoate is now prepared synthetically it occurs naturally in balsam of Peru, which has been employed as a scabicide in the past. In 1937 Kissmeyer[39] reported the effectiveness of benzyl benzoate in the treatment of 8000 cases of scabies, and its use rapidly became popular thereafter.[38,40–42] Most suggested treatment regimes involve two or three applications of a 25% emulsion, either within a 24-h period, on successive days, or with each application separated by an interval of a week. From several early reports of the use of benzyl benzoate it would appear that a single application washed off after 24 h should be effective in the majority of cases. However, the author is not aware of any published comparative trials of treatment regimes employing benzyl benzoate, a rather surprising situation in view of the length of time it has been in use. Benzyl benzoate is irritant, and patients should be warned about over-use.

Gamma benzene hexachloride (lindane)

A single application washed off after 24 h is usually recommended, but Taplin *et al.*[43] in a comparative trial of treatment schedules demonstrated an equivalent cure rate following 12-h and 6-h applications. Lindane is absorbed through the skin, and absorption occurs more readily if the barrier function of the epidermis is compromised.[44–47] There are a number of reports in the literature of adverse neurological effects, principally seizures, attributed to lindane,[46–50] but in the majority of these reports toxicity was associated with excessively lengthy application times, frequent repeated applications, use on infants whose epidermal barrier function was compromised by co-existent skin disease, or accidental ingestion. Kramer *et al.*,[51] using an algorithm developed to assist in the assessment of adverse drug reactions, analysed 26 reports of toxicity allegedly due to 1% gamma benzene hexachloride and considered that none scored as definite, and only six as probable adverse drug reactions. Of the six probable cases, five represented inappropriate application or unintentional ingestion. There have been only three reports to the Committee on Safety of Medicines in the U.K. of adverse neurological effects attributed to lindane since 1964. Critical assessment of the use of lindane as a scabicide suggests that if employed correctly it is safe, and adverse reactions are extremely rare.[52-54]

Monosulfiram (Tetmosol)[55,56]

A 25% solution in industrial methylated spirit is diluted with two or three parts of water to form an emulsion immediately prior to application, as the resulting suspension is not stable. It should be applied once daily on 2 or 3 consecutive days. Monosulfiram is chemically similar to disulfiram (Antabuse), and a disulfiram-like effect with flushing, sweating and tachycardia may occur if alcohol is ingested during, or soon after treatment.[57] Burgess[58] has suggested that this reaction may occur in the absence of oral ingestion of alcohol, probably as a response to the alcohol base of the preparation.

Monosulfiram-impregnated soap has been used as a prophylactic measure in communities where scabies was endemic,[59-62] and is still available as Tetmosol soap tablets (ICI).

Malathion[63,64]

Malathion 0·5% in an aqueous base is marketed in the U.K. as Derbac-M liquid, for the treatment of louse infection and scabies. In the treatment of scabies the preparation should be left on the skin for 24 h. A higher cure rate is obtained if a second treatment is applied after an interval of a few days.

Permethrin

Although not yet available in the U.K., 5% permethrin cream (Elimite) employed as a single application, washed off after 8–12 h, is reported as an effective scabicide.[65-67]

Other agents which have been used in the treatment of scabies include topical thiabendazole,[68,69] and crotamiton.[70] Both have limited scabicidal activity, and several applications on consecutive days are required to achieve significant cure rates.

In addition to the potential adverse effects of topical scabicides mentioned previously, contact dermatitis to mesulphen, used as a scabicide in Italy, has been reported by Meneghini *et al.*,[71] and a case of toxic epidermal necrolysis attributed to monosulfiram by Copeman.[72]

Scabicides are most conveniently applied by someone other than the patient, using a 5-cm paint brush. All areas of the body except the head and neck should be treated. Patients should be provided with instructions explaining their treatment regime, and warning against excessive use of the scabicide. They should be advised that itching will persist for a few days after treatment, but usually resolves completely within 2 weeks. A topical antipruritic such as crotamiton cream may be used on residual itchy areas. All members of the family and close physical contacts should also be treated, whether symptomatic or not. Treatment of clothing and bedding, other than by ordinary laundering, is not necessary.

Secondary infection should be treated with a systemic antibiotic. If secondary eczema is severe a non-irritant scabicide should be used.

Treatment of infants and young children

The irritant effects of benzyl benzoate, and the potential neurotoxicity of lindane have led many authors in the past to recommend avoidance of these preparations in infants. There has been much advice given about what not to use, but very little, or none at all, about which preparations are suitable, thus leaving the practitioner in a quandry. Benzyl benzoate's irritancy is usually transient, unless over-used, and this effect may be lessened by dilution with two or three parts water if it is to be used to treat infants. Prolonged application times, and frequent, repeated applications of lindane should certainly be avoided in children, and as a 6-h application time appears to be therapeutically effective it is probably preferable to employ this shorter application time when treating infants.

Monosulfiram and aqueous malathion also appear to be useful in the treatment of scabies in children. Crotamiton cream may be used to treat burrows on the head and neck in infants.

Scabicides in pregnancy

There is little authoritative guidance relating to the use of scabicides in pregnant women, nor are there any adequate and well-controlled studies on the use of scabicides in pregnancy. In animals, very high doses of lindane cause an increase in stillbirths, but not teratogenesis. The Committee on Safety of Medicines has received one report of phocomelia after treatment of scabies with lindane during the first trimester of pregnancy, but it is not clear whether lindane was the cause of the abnormality.[73] The uncertainty about toxic effects of lindane has led to suggestions that it should be used 'with caution' in pregnancy. As Rasmussen[54] points out 'use with caution' in this context is meaningless, as the preparation is either used or is not. The safety of other available scabicides in human pregnancy has not been assessed, but as far as the author is aware there have been no reports of adverse effects. In the absence of any evidence to the contrary it would appear to the author that there is no absolute contraindication to the use of any of the currently available scabicides in pregnancy.

Crusted (Norwegian) scabies

The treatments used in ordinary scabies are usually effective in crusted scabies, although several applications of a scabicide are often necessary. In a particularly recalcitrant case methotrexate has been used as part of the treatment regime.[74]

Management of institutional outbreaks of scabies

There are numerous reports of outbreaks of scabies affecting both patients and medical personnel in hospitals and residential homes for the elderly. If this type of situation is encountered all the patients on an affected ward, or all occupants of long-stay residential accomodation should be examined to establish whether there is a case of severe scabies amongst them who may have initiated the outbreak. Unless this is done it is quite likely that the problem will persist, in spite of what is usually considered effective anti-scabies therapy. If a patient suffering from severe or crusted scabies is discovered they should be isolated until effectively treated. Any medical or nursing personnel coming into contact with such a case should wear long-sleeved gowns and gloves. All individuals on an affected ward, or in a residential home, and all medical and nursing staff and their families should be treated with a scabicide. Bedding should be laundered, and communal areas such as sitting-rooms sprayed with an acaricide as an added precaution.[75–77] It has been suggested by Scher[78] that during treatment of institutional outbreaks of scabies particular attention should be paid to the nails, as mites may persist in a subungual location.

References

1 Altschuler DZ, Kenney LR. Pediculicide performance, profit and the public health. *Arch Dermatol* 1986; **122:** 259–61.
2 Meinking TL, Taplin D, Kalter DC, Eberle MW. Comparative efficacy of treatments for pediculosis capitis infestations. *Arch Dermatol* 1986; **122:** 267–71.
3 Rasmussen JE. Pediculosis: treatment and resistance. In. *Advances in Dermatology* Vol. 1. Chicago: Year Book Medical Publishers, 1986; 109–25.
4 Maunder JW. Resistance to organochlorine insecticides in head lice and trials using alternative compounds *Medical Officer* 1971, **125:** 27–9.
5 Maunder JW. Clinical and laboratory trials employing carbaryl against the human head louse, *Pediculus humanus capitis* (de Geer) *Clin Exp Dermatol* 1981; **6:** 605–12.
6 Maunder JW Use of malathion in the treatment of lousy children. *Community Medicine* 1971; **126:** 145–7.
7 Taplin D, Castillero PM, Spiegel J *et al.* Malathion for treatment of *Pediculus humanus* var *capitis* infestation. *JAMA* 1982; **247:** 3103–5.
8 Urcuyo FG, Zaias N. Malathion lotion as an insecticide and ovicide in head louse infestation. *Int J Dermatol* 1986; **25:** 60–2.
9 Burgess I. Carbaryl lotions for head lice—new laboratory tests show variations in efficacy. *The Pharmaceutical Journal* 1990; **245:** 159–61.
10 Treating head lice. *The Pharmaceutical Journal* 1989; **242:** 312.
11 Taplin D, Meinking TL. Pyrethrins and pyrethroids in dermatology. *Arch Dermatol* 1990, **126:** 213–21.
12 Taplin D, Meinking TL, Castillero PM, Sanchez R. Permethrin 1% creme rinse for the treatment of *Pediculus humanus* var *capitis* infestation. Pediatr Dermatol 1986; **3:** 344–8.
13 Carson DS, Tribble PW, Weart CW. Pyrethrins combined with piperonyl butoxide (RID) vs 1% permethrin (NIX) in the treatment of head lice. *Am J Dis Child* 1988; **142:** 768–9.
14 Brandenburg K, Deinard AS, DiNapoli J *et al.* 1% permethrin cream rinse vs 1% lindane shampoo in treating pediculosis capitis. *Am J Dis Child 1986;* **140:** 894–6.
15 Bowerman JG, Gomez MP, Austin RD, Wold DE. Comparative study of permethrin 1% creme rinse and lindane shampoo for the treatment of head lice. *Pediatr Infect Dis J* 1987; **6:** 252–5.
16 DiNapoli JB, Austin RD, Englender SJ *et al.* Eradication of head lice with a single treatment. *Am J Public Health* 1988; **78:** 978–80.
17 Shashindran CH, Gandhi IS, Krishnasamy S, Ghosh MN, Oral therapy of pediculosis capitis with cotrimoxazole. *Br J Dermatol* 1978; **98:** 699.
18 Campos R, Moreira AAB, Castilho VLP *et al.* Cura da pediculosa da cabeça por meio do cotrimoxazol administrado pela via oral. *Rev Inst Med trop São Paulo* 1981; **23:** 28–30.
19 Burns DA. Action of cotrimoxazole on head lice. *Br J Dermatol* 1987; **117:** 399–400.
20 Karacic I, Yawalkar SJ. A single application of crotamiton lotion in the treatment of patients with pediculosis capitis. *Int J Dermatol* 1982; **21:** 611–13.
21 DeFelice J, Rumsfield J, Bernstein JE, Roshal JY. Clinical evaluation of an after-pediculicide nit removal system. *Int J Dermatol* 1989; **28:** 468–70.
22 Maunder JW. Pediculosis corporis; an updating of attitudes. *Environ Health* 1983; May: 130–2.
23 Hayes WJ Jr, Mattson AM, Short JG, Witter RF Safety of malathion dusting powder for louse control. *Bull WHO* 1960; **22:** 503–14.
24 Barnes WW, Eldridge BF, Greenberg JH, Vivona S. A field evaluation of malathion dust for the control of body lice. *J Econ Entomol* 1962; **55:** 591–4

25 Scholdt LL, Rogers EJ Jr, Gerberg EJ, Schreck CE. Effectiveness of permethrin-treated military uniform fabric against human body lice. *Milit Med* 1989; **154:** 90–3.

26 Smith DE, Walsh J. Treatment of pubic lice infestation: A comparison of two agents. *Cutis* 1980; **26:** 618–9.

27 Orkin M, Maibach HI. Treatment of today's pediculosis. In: *Cutaneous Infestations and Insect Bites*. (Orkin M, Maibach HI, eds). New York: Marcel Dekker, 1985: 213–7.

28 Kalter DC, Sperber J, Rosen T, Matarasso S. Treatment of pediculosis pubis. *Arch Dermatol* 1987; **123:** 1315–9.

29 Ronchese F. Treatment of pediculosis ciliorum in an infant. *N Engl J Med* 1953; **249:** 897–8.

30 Couch JM, Green WR, Hirst LW, De La Cruz ZC. Diagnosing and treating *Phthirus pubis* palpebrarum. *Surv Opthalmol* 1982; **26:** 219–25.

31 Burns DA. The treatment of *Pthirus pubis* infestation of the eyelashes. *Br J Dermatol* 1987; **117:** 741–3.

32 Awan KJ. Cryotherapy in phthiriasis palpebrarum. *Am J Ophthalmol* 1977; **83:** 906–7.

33 Mathew M, D'Souza P, Mehta DK. A new treatment of pthiriasis palpebrarum. *Ann Opthalmol* 1982; **14:** 439–41.

34 Rasmussen JE. Pediculosis and the pediatrician. *Pediatr Dermatol* 1984; **2:** 74–9.

35 Duke-Elder S, MacFaul PA. *System of Ophthalmology Vol. X11 The Ocular Adnexa. Part 1 Diseases of the Eyelids*. London: Henry Kimptom, 1974: 196–9.

36 Chin GN, Denslow GT. Pediculosis ciliaris. *J Pediatr Ophthalmol Strabismus* 1978; **15:** 173–5.

37 Alexander JO'D. Scabies. In: *Arthropods and Human Skin*. Berlin: Springer-Verlag, 1984: 227–92.

38 Mellanby K, Johnson CG, Bartley WC. The treatment of scabies. *Br Med J* 1942; **2:** 1–4.

39 Kissmeyer A. Rapid ambulatory treatment of scabies with benzyl benzoate lotion. *Lancet* 1937; **i:** 21.

40 King RE. The benzyl benzoate treatment of scabies. *Br Med J* 1940; **2:** 626–7.

41 Mackenzie IF. Scabies treated by a benzyl benzoate emulsion. *Br Med J* 1941; **2:** 403–5.

42 Graham JR. Scabies treated with one application of benzyl benzoate. *Br Med J* 1943; **1:** 413–4.

43 Taplin D, Rivera A, Walker JG, *et al*. A comparative trial of three treatment schedules for the eradication of scabies. *J Am Acad Dermatol* 1983; **9:** 550–4.

44 Ginsburg CM, Lowry W, Reisch JS. Absorption of lindane (gamma benzene hexachloride) in infants and children. *J Pediatr* 1977; **91:** 998–1000.

45 Solomon LM, Fahrner L, West DP. Gamma benzene hexachloride toxicity. *Arch Dermatol* 1977; **113:** 353–7.

46 Pramanik AK, Hansen RC. Transcutaneous gamma benzene hexachloride absorption and toxicity in infants and children. *Arch Dermatol* 1979; **115:** 1224–5.

47 Friedman SJ. Lindane neurotoxic reaction in non-bullous congenital ichthyosiform erythroderma. *Arch Dermatol* 1987; **123**, 1056–8.

48 Lee B, Groth P. Scabies: transcutaneous poisoning during treatment. *Pediatrics* 1977; **59:** 643.

49 Telch J, Jarvis DA. Acute intoxication with lindane (gamma benzene hexachloride). *Can Med Assoc J* 1982; **126:** 662–3.

50 Davies JE, Dedhia HV, Morgade C *et al*. Lindane poisonings. *Arch Dermatol* 1983; **119:** 142–4.

51 Kramer MS, Hutchinson TA, Rudnick SA *et al*. Operational criteria for adverse drug reactions in evaluating suspected toxicity of a popular scabicide. *Clin Pharmacol Ther* 1980; **27:** 149–55.

52 Rasmussen JE. The problem of lindane. *J Am Acad Dermatol* 1981; **5:** 507–16.

53 Shacter B. Treatment of scabies and pediculosis with lindane preparations: An evaluation. *J Am Acad Dermatol* 1981; **5:** 517–27.

54 Rasmussen JE. Lindane. A prudent approach. *Arch Dermatol* 1987; **123:** 1008–10.

55 Clayton TM. Treatment of scabies by T.E.T.M.S. *Br Med J* 1943; **1:** 443–5.

56 Bradshaw DB. Tetraethylthiuram monosulphide in the treatment of scabies. *Lancet* 1944; **ii:** 273–4.

57 Gold S. A skinful of alcohol. *Lancet* 1966; **ii:** 1417.

58 Burgess I. Adverse reactions to monosulfiram. *Lancet* 1990; **336:** 873.

59 Gordon RM, Davey TH, Unsworth K *et al*. Control of scabies by use of soap impregnated with tetra-ethylthiuram monosulphide ('Tetmosol'). *Br Med J* 1944; **1:** 803–6.

60 Bartley W, Unsworth K, Gordon RM. The rise in incidence of scabies in a closed community using ordinary soap, and its subsequent fall on the substitution of 5% Tetmosol soap. *Br Med J* 1945; **1:** 332–3.

61 Mellanby K. Scabies prophylaxis using 'Tetmosol' soap. *Br med J* 1945; **1:** 38–9.

62 Reid HF, Thorne CD. Scabies Infestation: the effect of intervention by public health education. *Epidemiol Infect* 1990; **105:** 595–602.

63 Hanna NF, Clay JC, Harris JRW. Sarcoptes scabiei infestation treated with malathion liquid. *Br J Vener Dis* 1978; **54:** 354.

64 Burgess I, Robinson RJF, Robinson J *et al*. Aqueous malathion 0·5% as a scabicide: a clinical trial. *Br Med J* 1986; **292:**1172.

65 Taplin D, Meinking TL, Porcelain SL *et al*. Permethrin 5% dermal cream: a new treatment for scabes. *J Am Acad Dermatol* 1986; **15:** 995–1001.

66 Van Der Rhee HJ, Farquhar JA, Vermeulen NPE. Efficacy and transdermal absorption of permethrin in scabies patients. *Acta Derm Venereol (Stockh)* 1989; **69:** 170–82.

67 Schultz MW, Gomez M, Hansen RC *et al*. Comparative study of 5% permethrin cream and 1% lindane lotion for the treatment of scabies. *Arch Dermatol* 1990; **126:** 167–70.

68 Biagi F, Delgado-Y-Garnica R. First therapeutic trials in the treatment of scabies with thiabendazole cream. *Int J Dermatol* 1974; **13:** 102–3.

69 Hernandez-Perez E. Topically applied thiabendazole in the treatment of scabies. *Arch Dermatol* 1976; **112:** 1400–1.

70 Cubela V, Yawalkar SJ. Clinical experience with crotamiton cream and lotion in the treatment of infants with scabies. *Br J Clin Pract* 1978; **32:** 229–31.

71 Meneghini CL, Vena GA, Angelini G. Contact dermatitis to scabicides. *Contact Dermatitis* 1982; **8:** 285–6.

72 Copeman PWM. Toxic epidermal necrolysis caused by skin hypersensitivity to monosulfiram. *Br Med J* 1968; **1:** 623–4.

73 Anon. Treating scabies. *Drug Ther Bull* 1988; **26:** 19–20.

74 Ward WH. Scabies Norvegica. Treatment with methotrexate. *Aust J Dermatol 1971;* **12:** 44–51.

75 Carslaw RW, Dobson RM, Hood AJK, Taylor RN. Mites in the environment of cases of Norwegian scabies. *Br J Dermatol* 1975; **92:** 333–7.

76 Lerche NW, Currier RW, Juranek DD *et al*. Atypical crusted 'Norwegian' scabies: report of nosocomial transmission in a community hospital and an approach to control. *Cutis* 1983; **31:** 637–84.

77 Burns DA. An outbreak of scabies in a residential home. *Br J Dermatol* 1987; **117:** 359–61.

78 Scher RK. Subungual scabies. *Am J Dermatopathol* 1983; **5:** 187–9.

British Journal of Dermatology (1991) 125, 94–100. ADONIS 000709639100177J

The DEBR rat: an animal model of human alopecia areata

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Accepted for publication 30 January 1991

Summary

The Dundee experimental bald rat (DEBR) is reported as a model for human alopecia areata. Parallels with human alopecia areata were observed in relation to the gradual and patchy loss of hair and the penetration of both pelage and vibrissa follicles by mononuclear cells. In particular, the apparent disruption of the follicles within the precortical region of the epidermal component and consequent alterations of normal geometrical relationships between dermal and epidermal components relate directly to similar studies on human alopecia areata. In comparison with other previously described hypotrichotic rodent mutants, the DEBR rat exhibits a unique mechanism of hair loss which may provide important information regarding the pathomechanism of human alopecia areata.

Animal strains which spontaneously develop conditions reflecting human disease have immense potential as models, for investigating the pathogenesis of the disease and for evaluating therapies to alleviate or to reverse the condition.

Many mammalian species exhibit hereditary hair loss,[1] but the greatest frequency of mutations causing hair growth defects occurs in inbred colonies of rodents. Because rodents are the most extensively studied animals from all aspects of hair research, these mutants have been the focus of a considerable amount of work. Two reports exist of hamsters showing hair abnormalities[2] and one of guinea-pigs[3] but in mice and rats many different mutations have occurred. Included among mouse mutants showing defects in hair production are: hairless (hr),[4] naked (n),[1] rhino (rh),[5] crinkled (cr)[6] and nude (*nu*).[7] In rats, such mutations are apparently less numerous but the following examples have been described: hairless (hr), naked (n), fuzzy (fz),[8] nude (*nu*)[9] and bald (ba).[10] To date, however, none of the histological observations on the skin of the above named rodent mutants have described a mononuclear cell infiltrate surrounding and penetrating the hair follicles. This association of hair loss with immunological attack is characteristic of the human condition of hair loss, alopecia areata.

We propose a strain of rat, the Dundee experimental bald rat (DEBR) whose hair loss has been found to parallel many aspects of alopecia areata including cellular invasion of the follicle, as an investigative model for alopecia areata. The DEBR rat has been bred at the University of Dundee since March 1984. The original crosses involved the inbred stock of BD1X rats showing the lesion held at the University of Dundee and Wistar rats. The descendants of this cross resulted from full sib-matings. No abnormal mortality is exhibited and litter sizes number, on average, nine pups. Two strains of DEBR rat are being developed: the black-hooded and brown-hooded strains.

Methods

Animals and immobilization

For the current studies DEBR rats of the brown-hooded strain of both sexes were used from the F1 generation. Animals were caged separately according to sex and maintained on a diet of expanded pellets (SDS Ltd) and tap-water *ad libitum*. Ambient conditions were as follows: temperature 22°C, relative humidity 50%, 12 h of light, 12 h of darkness.

For investigative procedures animals were immobilized by an intramuscular injection of Hypnorm (Janssen Pharmaceutical Ltd; 0·05ml/100g body weight).

Development of the lesion

Macroscopic changes. A longitudinal study was carried out on 24 rats from three litters of both sexes. The study began at birth until an age when it was judged that the lesion had run its full course for any one particular

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animal. The results from this study were recorded photographically.

In addition, four litter-mate rats, three females and one male, were clipped from an age of 2 weeks with small blunt-ended scissors in an area between the pectoral and pelvic girdles, on both dorsal and ventral surfaces, by cutting close to but without damaging the skin. The same area was re-clipped when regrowth occurred. Time intervals between successive hair removals varied between 1 and 3 weeks depending on the amount of growth. By using this method the cyclical progression of hair replacement was clearly revealed.

Microscopic changes. For routine light microscopy, individual vibrissa follicles and skin biopsies were taken from 37 animals at different stages of the lesion. Skin biopsies were taken from the neck, abdomen and flank. Control biopsy material was obtained from PVG/C rats (colony, University of Dundee). All material was fixed in formal saline immediately after removal for a minimum period of 2 days, followed by dehydration through an ascending series of ethanol, clearing in Histoclear (National Diagnostics) and embedding in paraffin wax at 60°C before serially sectioning the biopsies at 8 μm parallel to the longitudinal axis of the follicles. Sections were stained with Harris' haematoxylin and eosin.

Results

External features of lesional development

At birth, the DEBR rats were naked apart from the presence of very fine vibrissae. After 5 days the first hairs of the pelage erupted on the dorsum. Eruption of the

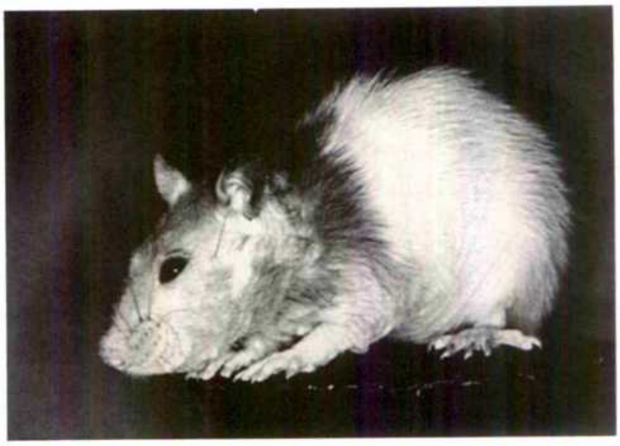

Figure 2. Actively lesional brown-hooded, female DEBR rat aged 6 months. Hair loss has occurred on the ventral surface and is spreading over the head and on the sides of the rat. Many of the vibrissa fibres have been lost.

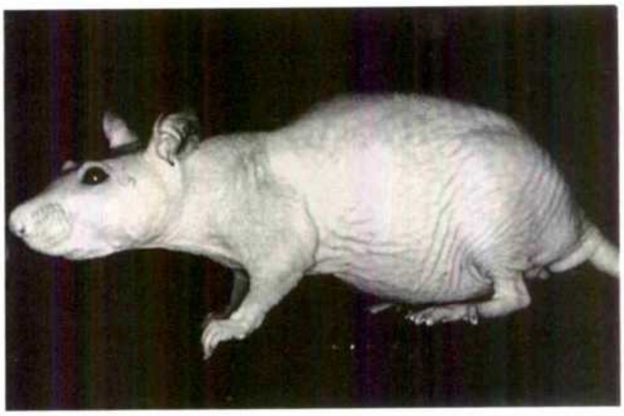

Figure 3. Brown-hooded, female DEBR rat aged 1 year. Total loss of pelage hairs and vibrissa fibres has occurred. Note the folding of the skin on the ventrum.

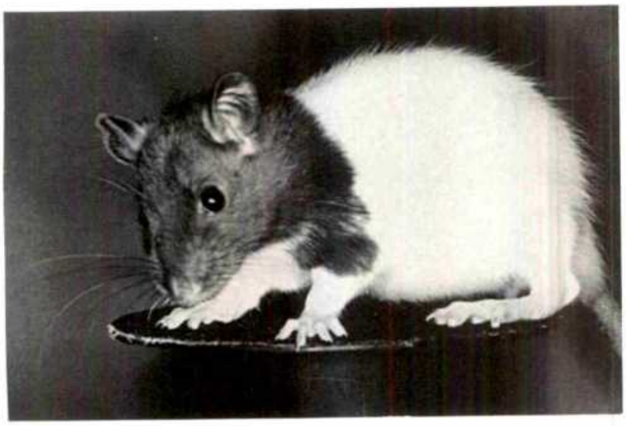

Figure 1. Pre-lesional brown-hooded, male DEBR rat aged 2 months with full pelage.

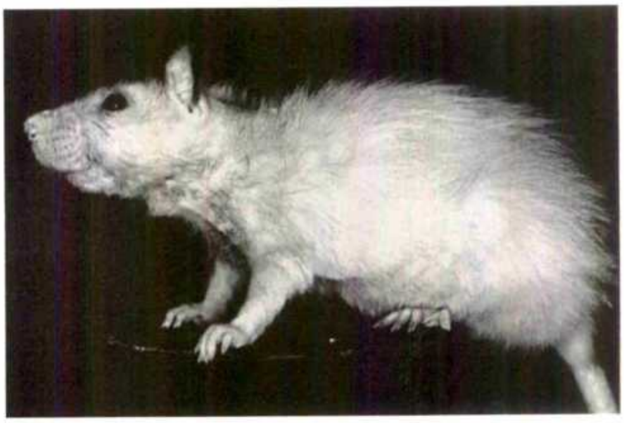

Figure 4. Brown-hooded female DEBR rat aged 8 months. A general thinning of the hair has occurred over the body surface resulting in a 'staring' coat.

ventral hairs did not occur until the tenth day. A full pelage was apparent by 2 weeks and was retained (Fig. 1) until an age of 3·5–4 months when the lesion was first observed. In pigmented areas the onset of lesional activity was associated with a preceding loss of pigment from the hairs. Skin abrasions, taken as evidence of scratching, were often observed in areas which had undergone hair loss.

The first area to exhibit hair loss was the ventral surface of the hind legs. The rapid spread of the lesion to the abdomen and thorax coincided with lesional expression on the snout including the vibrissae. Loss of hair on the snout proceeded towards the eyes and ears (Fig. 2) and continued backwards over the neck to coalesce with hair loss on the sides from the abdomen. Meanwhile the lesion at the rear of the animal spread over the flanks to merge with hair loss which had begun anteriorly. The process of hair loss was gradual over 3–4 months so that by 8 months substantial hair loss could be seen. Due to normal differences in hair densities between different regions of the body, hair loss in some regions tended to outstrip the loss of hair in other regions. Complete hair loss was attained by one in 20 rats (Fig. 3) and was often associated with folding of the skin, especially on the ventral surface. Many of the totally bald rats showed intense pigmentation of the skin over the head and neck where pigmented hair had previously been produced.

A variation in the above pattern of hair loss was a general uniform thinning of the hair over the entire body surface. This pattern, in common with the major pattern of hair loss described previously, showed retention of the larger guard hairs but over a wider area resulting in a 'staring' coat (Fig. 4). A small number of rats which exhibited large amounts of hair loss, spontaneously regrew hair. Moreover, much variation in the intensity of lesional expression occurred between litter mates. The female rats lost their hair more rapidly and to a greater extent over the body surface than the male rats. The minimum expression of the lesion involved a slight greying and thinning of the hair on the head including the vibrissae and thinning of the hair on the ventral surface of the hind legs.

When the pelage was clipped it was observed that waves of hair growth progressed from the mid-ventrum to the mid-dorsum. Lesional loss of hair began on the fourth generation of pelage hair, i.e. the third replacement generation, and was clearly observed on the ventral surface. Hair replacement during this cycle was sparse, indicating that many of the hair follicles only underwent three cycles before lesional activity disrupted hair production.

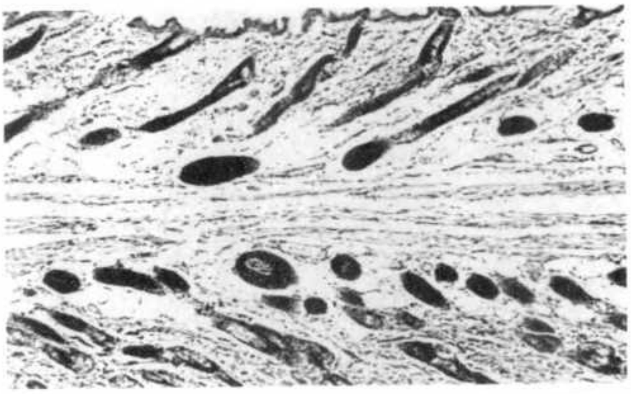

Figure 5. Longitudinal section of a flank skin biopsy from a DEBR rat aged 3 weeks. The hair follicles are in anagen (× 65).

Histological observations on lesional development

The progression of the hypotrichotic lesion in the DEBR rats appeared to follow similar steps within both the vibrissa and pelage follicles.

Biopsies obtained from 3-week-old DEBR and PVG/C (control) rats exhibited comparable follicular morphologies (Fig. 5), showing well-formed dermal papillae and

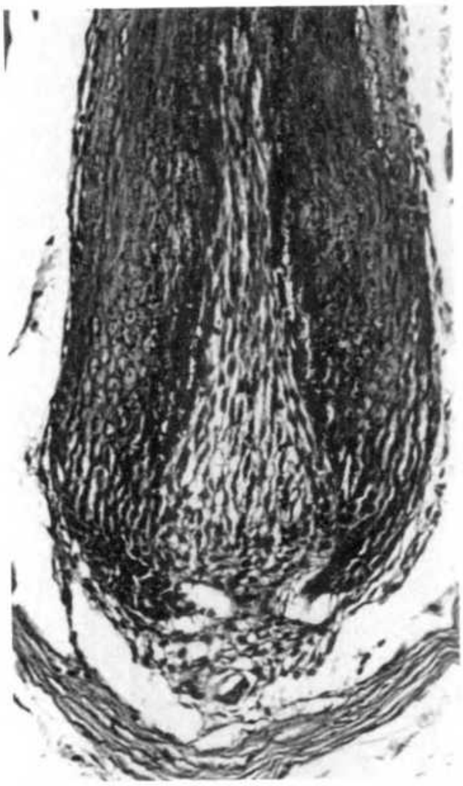

Figure 6. Longitudinal section of a pre-lesional vibrissa follicle in anagen showing the extension of the papillary apex into the epidermal component (× 170).

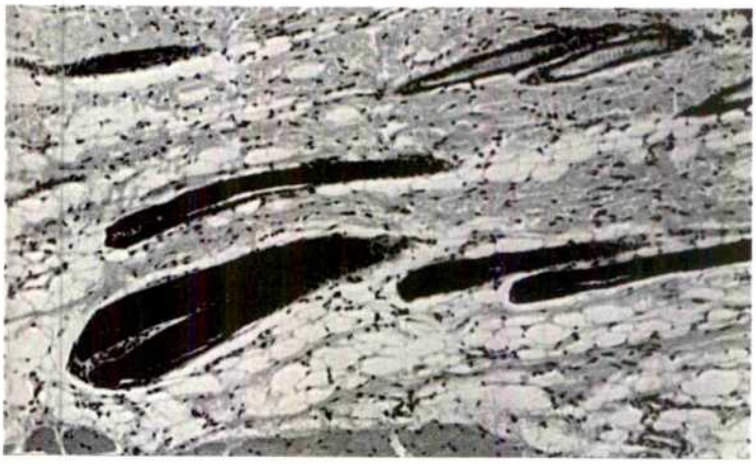

Figure 7. Longitudinal section of abdominal skin from a DEBR rat aged 8 weeks. Note the presence of mononuclear cells within the inter-follicular dermis. The follicles are in anagen (×65).

differentiated epidermal matrices. A prominent feature of the pre-lesional vibrissa follicle was its dermal papilla, the apex of which extended deep into the epidermal matrix during anagen (Fig. 6).

Between the ages of 8 and 10 weeks the first signs of lesional activity became apparent. An increased number of mononuclear cells was observed in the dermis of skin biopsies (Fig. 7) and within the blood sinus region, especially at the bases of the dermal papillae, of vibrissa follicles (Fig. 8). At approximately 14 weeks the mononuclear cells became more numerous, the follicles were penetrated and the follicular epidermis disrupted at the apical pole of the dermal papilla (Fig. 9). As the lesion progressed the intraepidermal infiltrate was associated with disruption, damage and cell death within the precortical area of the epidermal matrix and with non-production of hair. In all biopsy sites the majority of follicles appeared to be arrested in a state of dystrophic anagen for long periods of time with continued peri- and intrafollicular presence of mononuclear cells. In areas of pigmented skin, clusters of melanin granules were observed within the dermis at the bases of some infiltrated follicles. Later skin biopsies revealed varying levels of mononuclear cell presence apparently dependent on the cyclical morphology of the follicles (Fig. 10). Vibrissa follicles appeared to linger in a state of late anagen without follicular involution and with the epidermal:dermal ratio often much reduced (Fig. 11). Considerable variation was observed in lesional intensity between individual follicles and their associated disruption of normal morphology.

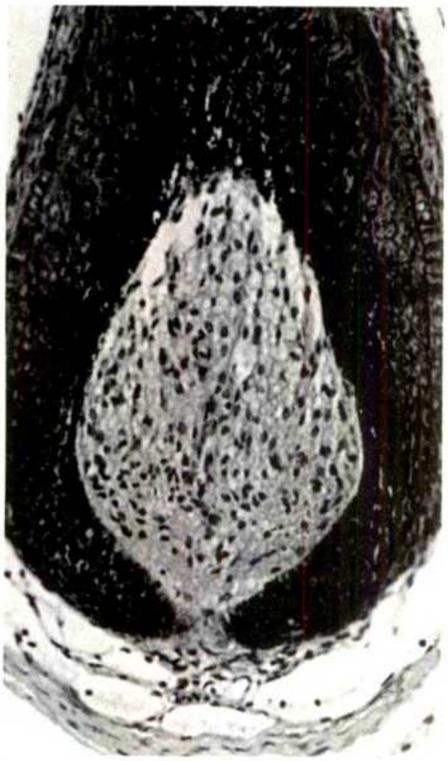

Figure 8. Longitudinal section of DEBR rat vibrissa follicle at 10 weeks. Mononuclear cells are present within the blood sinus, especially at the base of the dermal papilla (×170).

Discussion

Our study shows that both macroscopically and histologically the DEBR rats show a marked contrast with previously described hypotrichotic mutants and exhibit many of the basic features seen in alopecia areata.

Macroscopically, the initial normality of both whiskers and pelage during the first 3 months of age contrasts with other hair-losing mutants[8,10] where hair is lost rapidly and almost totally. The pattern of pelage replacement in the DEBR rat was clearly revealed by clipping the coat. Waves of hair replacement passed from the ventrum to dorsum in a sequence consistent with

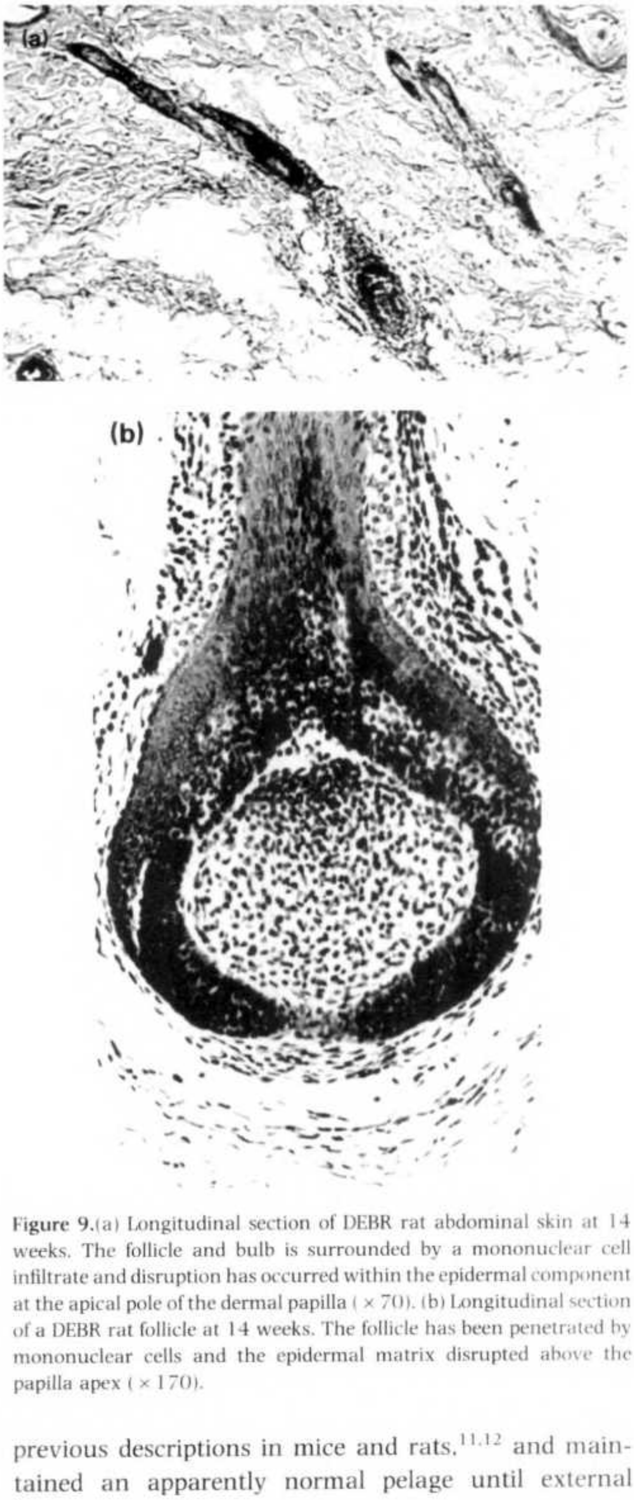

Figure 9.(a) Longitudinal section of DEBR rat abdominal skin at 14 weeks. The follicle and bulb is surrounded by a mononuclear cell infiltrate and disruption has occurred within the epidermal component at the apical pole of the dermal papilla (× 70). (b) Longitudinal section of a DEBR rat follicle at 14 weeks. The follicle has been penetrated by mononuclear cells and the epidermal matrix disrupted above the papilla apex (× 170).

previous descriptions in mice and rats,[11,12] and maintained an apparently normal pelage until external lesional expression.

The active lesion was initially manifested with loss of hair pigment in the pigmented areas of the pelage. The resulting greyness may be due to a primary initial sensitivity of the pigmentary system of the hair follicles to the lesion whilst hair growth was maintained.

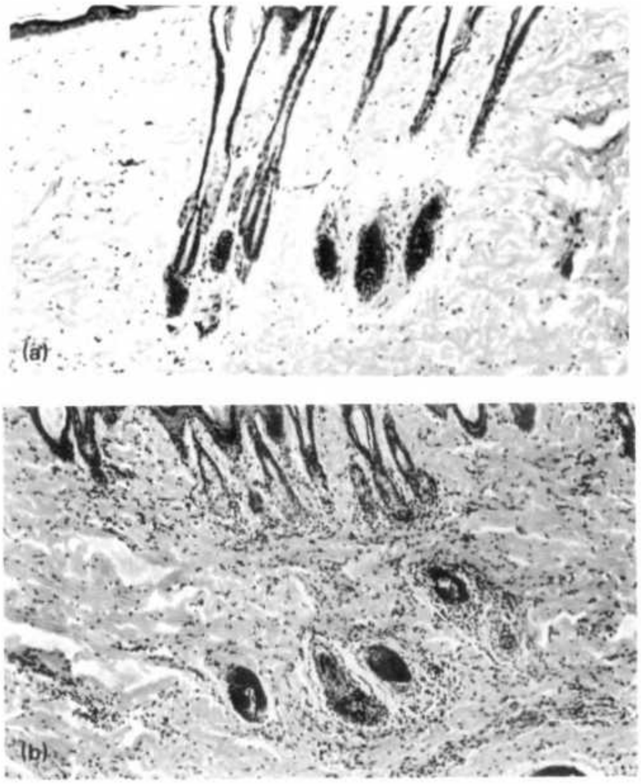

Figure 10. (a) Longitudinal section of abdominal skin biopsy at 23 weeks showing normal follicular involution associated with a marked reduction of mononuclear cell infiltrate. (b) Longitudinal section of DEBR rat flank biopsy at 9 months showing a resurgence in density of leucocytic infiltrate associated with anagen follicles.

Associations between human alopecia areata and pigmentation have been found in cases of vitiligo[13,14] and ocular abnormalities.[15] Messenger and Bleehen[16] observed that in alopecia areata some individuals showed sparing of senile white hairs whilst the pigmented hairs are lost, indicating that the latter may be in some way more susceptible to lesional activity. The intense pigmentation which many of the totally bald rats exhibited indicated that the pelage follicles, at least within the head and neck regions, were in the anagen phase of the hair cycle.

The pattern of lesional loss of hair over the body surface of the DEBR rats was gradual, symmetrical and from the ventral to dorsal surfaces and thus followed the direction of pelage replacement. In contrast, the hair loss of other rodent mutants occurs rapidly and in the anterio-posterior direction.[17,18] Where the hair thinning process of the DEBR rats led to an almost complete loss of hair in any region of skin, this was reminiscent of human alopecia areata which is defined as the loss of hair within a well-circumscribed and clearly defined round or oval patch in a non-scarring manner.[19]

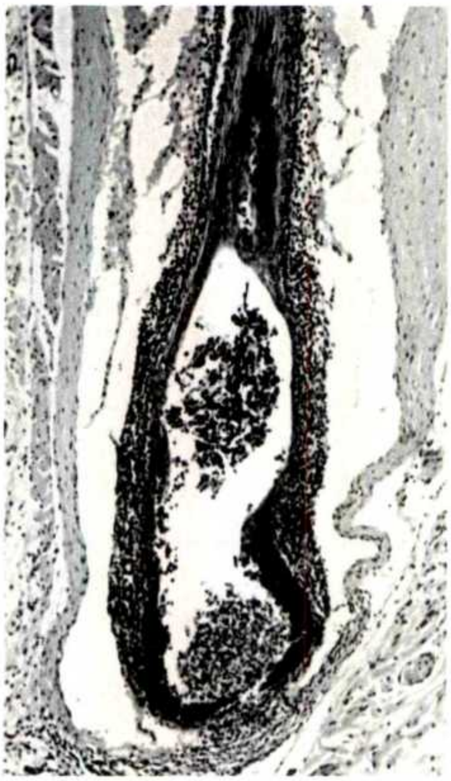

Figure 11. Longitudinal section of DEBR rat vibrissa follicle at 23 weeks in anagen. The normal epidermal: dermal ratio is much reduced and the dermal papilla is condensed. Cellular debris and melanin granules are present within the precortex (× 170).

The lesion in the DEBR rats exhibited heterogeneity between litter mates and between the sexes and may in this respect reflect the heterogeneity as exhibited by alopecia areata in man. In some patients a regrowth of hair can occur with or without recurrence of hair loss. In others it may spread to involve the rest of the scalp (alopecia totalis) or all the body hair (alopecia universalis).

The normality of the pre-lesional state of the DEBR rat pelage was reflected in the histological appearance of both vibrissa and pelage follicles, which was described for normal-haired strains.[20,21] It was apparent that the breakdown of hair production was related to the appearance of the mononuclear cells around the hair follicles. Such an infiltrate has not been previously reported for rodents, but a perifollicular infiltrate has been recorded for Appaloosa and Palomino horses which lose hair[22] and is also a characteristic of alopecia areata in man.[23]

The vibrissa follicles provided an illuminating model as they presented a magnified view of the development of the alopecic lesion in the DEBR rat. In these follicles leucocytic penetration via the dermal papilla clearly occurred. In human alopecia areata there was controversy regarding follicular penetration by lymphocytes,[16,23] but this has been resolved by more recent immunohistochemical studies which showed that the lymphocytes do penetrate the dermal papilla and bulbar epithelium.[24]

From histological and autoradiographic studies on human hair follicles[25,26] it has been ascertained that the matrix cells of the lower hair bulb form the inner root sheath, whilst those of the upper half of the matrix form the precortical cells. Assuming that these observations can be applied to the rat hair follicles, it would appear that the disruption and cell injury inflicted by the infiltrating leucocytes occurred within the precortical region of both types of follicle, thus causing the observed non-production of hair. This is in agreement with work carried out by Messenger and Bleehen[16] but contrasts with the earlier observations of Van Scott,[23] who highlighted degenerative changes of the connective tissues surrounding the intrapapillary blood vessels, division of the papilla cells, a breakdown of the ground substance and an invasion of the outer root sheath above the end bulb by the lymphocytes.

The biopsy material from the DEBR rats in the present study revealed that the majority of follicles appeared to linger in a state of dystrophic anagen whilst surrounded by the lymphocytic infiltrate and transformed to telogen when the inflammation abated. This suggests that the follicles of the DEBR rats may flip between anagen and telogen depending on the levels of infiltrating cells around them. It is widely accepted that in alopecia areata a breakdown of the normal follicular dynamics occurs but there is disagreement as to the stage of the cycle the follicle is forced into as a result of the insult.[27-29] The widely accepted view is that the follicles proceed into a state of premature telogen.[27,28] However Messenger *et al.*[29] concluded that whilst some follicles transform to telogen after insult, others are retained in a dystrophic anagen state or may oscillate between telogen and anagen IV.

The geometrical relationship between the dermal and epidermal components within the DEBR rat follicles were grossly altered. This feature of lesional activity in the DEBR rat corresponds to human alopecia areata where a similar disproportionate reduction of the epidermal matrix over the dermal papilla has been observed.[29,30]

It is therefore apparent that the mechanism of hair loss in the DEBR rat is entirely different from any hypotrichotic rodent as yet described. The mononuclear cell infiltrate caused a disruption of normal follicular dynamics and defective fibre production without any detectable scar formation as seen using light microscopy. The results obtained so far at both the macroscopic and microscopic levels, and previous immunobiological results on the DEBR rat,[31] correlate well with descriptions of human alopecia areata. These findings indicate that the DEBR rat could prove to be an invaluable model for studying the aetiology and pathogenesis of alopecia areata and, most importantly, for developing therapies to alleviate the condition in man.

Acknowledgments

We are grateful to Mr B.F.Pert for the preparation of the photographs. This work was supported by the Medical Research Council. CABJ was a 1983 Royal Society University Research Fellow.

References

1 David L. The external expression and comparative dermal histology of hereditary hairlessness in mammals. *Z Zellforsch Mikro Anat* 1932; 14: 616–9.
2 Nixon C. Hereditary hairlessness in the Syrian golden hamster. *J Hered* 1972; 63: 215–7.
3 Reed C, O'Donoghue J. A new guinea pig mutant with abnormal hair production and immunodeficiency. *Lab Anim Sci* 1979; 29: 744–8.
4 Brooke H. Hairless mice. *J Hered* 1926; 17: 173–4.
5 Howard A. 'Rhino': an allele of hairless in the house mouse. *J Hered* 1940; 31: 467–70.
6 Falconer D, Fraser A, King J. The genetics and development of 'crinkled', a new mutant in the house mouse. *J Genet* 1951; 50: 324–44.
7 Flanagan S. 'Nude': a new hairless gene with pleiotropic effects in the mouse. *Genet Res* 1966; 8: 295–304.
8 Castle W, Dempster E, Shurrager H. Three new mutations of the rat. *J Hered* 1955; 46: 9–14.
9 Festing M, May D, Conners T *et al.* An athymic nude mutation in the rat. *Nature* 1978; 274: 365–6.
10 Inazu M, Sakaguchu T. Morphologic characteristics of the skin of bald mutant rats. *Lab Anim Sci* 1984; 34: 584–7.
11 Dry F. The coat of the mouse *Mus musculus*. *J Genet* 1926; 16: 287–340.
12 Ebling FJ, Johnson E. The control of hair growth. *Symp Zool Soc Lond* 1964; 12: 97–126.
13 Anderson I. Alopecia areata, a clinical study. *Br Med J* 1950; 2: 1250–2.
14 Muller S, Winkelmann R. Alopecia areata. *Arch Dermatol* 1963; 88: 290–7.
15 Brown AC, Pollard ZF, Jarret WH. Ocular and testicular abnormalities in alopecia areata. *Arch Dermatol* 1982; 118: 546–54.
16 Messenger AG, Bleehen SS. Alopecia areata: light and electron microscope pathology of the regrowing white hair. *Br J Dermatol* 1984; 110: 155–62.
17 Rigdon R. Hairlessness in *Mus musculus*. A pathologic study. *Arch Pathol* 1975; 99: 318–22.
18 Inazu M, Kasai K, Sakaguchi T. Characteristics of a new hairless mutant (bald) in rats. *Lab Anim Sci* 1984; 34: 577–83.
19 Mitchell A, Krull E. Alopecia areata: Pathogenesis and treatment. *J Am Acad Dermatol* 1984; 11: 763–75.
20 Gibbs H. A study of the post-natal development. *Anat Rec* 1941; 80: 61–81.
21 Oliver RF. Whisker growth after removal of the dermal papilla and lengths of the follicle in the hooded rat. *J Embryol exp Morph* 1966; 15: 331–47.
22 Conroy J. Alopecia areata. In: *Spontaneous Animal Models of Human Disease* (J Edwin, C Andrews, B Ward, N Allman, eds) Vol 2. New York: Academic Press, 1979; 30–1.
23 Van Scott E. Evaluation of disturbed hair growth in alopecia areata and other alopecias. *Ann NY Acad Sci* 1959; 83: 480–90.
24 Perret C, Wiesner-Menzel L, Happle R. Immunohistochemical analysis of T-cell subsets in the peribulbar and intrabulbar infiltrates of alopecia areata. *Acta Derm Venereol (Stockh)* 1984; 64: 26–30.
25 Kligman A. The human hair cycle. *J Invest Dermatol* 1959; 33: 307–16.
26 Epstein W, Maibach H. Cell proliferation and movement in human hair bulbs. In: *Advances in Biology of Skin* (W. Montagna, R. Dobson, eds), Vol. 9. Oxford: Pergamon Press, 1968, 83–97.
27 Eckert J, Church RE, Ebling FJ. The pathogenesis of alopecia areata. *Br J Dermatol* 1968; 80: 203–10.
28 Swanson NA, Mitchell AJ, Leahy MS *et al.* Topical treatment of alopecia areata. Contact allergen vs primary irritant therapy. *Arch Dermatol* 1981; 117: 384–7.
29 Messenger AG, Slater DN, Bleehen SS. Alopecia areata: alterations in the hair growth cycle and correlation with the follicular pathology. *Br J Dermatol* 1986; 114: 337–47.
30 Van Scott E, Ekel T. Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. *J Invest Dermatol* 1958; 31: 281–7.
31 Michie HJ, Jahoda CAB, Oliver RF, Poulton TA. Immunobiological studies on the alopecic (DEBR) rat. *Br J Dermatol* 1990; 123: 557–67.

British Journal of Dermatology (1991) **125**, 101–107.   ADONIS 000709639100178W

Detection of a novel basement membrane antigen by GDA-J/F3 anti-human sperm fibrous sheath monoclonal antibody

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Accepted for publication 27 February 1991

Summary Basement membrane zones (BMZ) of human epithelia were stained with GDA-J/F3 monoclonal antibody, which was originally raised against sperm cells. Using indirect immunofluorescence and immunoperoxidase techniques, the antibody reacted with the BMZ of stratified squamous epithelia (skin and its appendages, tongue, lip, oesophagus and cervix). It also stained the BMZ of trachea, nasal ciliated mucosa, some mammary ducts of lactating and resting breast, amnion and ureter but failed to react with that of stomach, ileum, colon, rectum, kidney, liver, fallopian tube, lung or their blood vessels. In testes, the antibody did not react with the BMZ of the seminiferous tubules although the sperm tails were stained. Split-skin immunofluorescence and immunoelectron microscopy localized GDA-J/F3 antigen to the inferior border of the lamina densa of the BMZ. In human foetuses, the epidermally associated antigen was detected at an estimated gestational age of 9 weeks, and in the amnion at 15 weeks. The antibody reacted with tissues from monkey but not from mouse, rat, cow or pig suggesting the late appearance of the antigen during evolution. Although the GDA-J/F3 was difficult to characterize biochemically, its tissue distribution, ontogeny and ultrastructural localization suggests that this antigen may be a type VII collagen-associated protein, whose expression is altered in recessive dystrophic epidermolysis bullosa. This disease could represent abnormalities in type VII collagen structure, assembly, transport or interaction with associated proteins.

Basement membrane zones (BMZ) are complex structures which separate epithelial and endothelial cells from underlying mesenchyme. Their functions are thought to include adhesion and stabilization, the control of permeability and nutrition of the epithelium and the direction of epithelial–mesenchymal interactions and epithelial differentiation, particularly during development. Ultrastructurally, the epidermal BMZ is made up of four components: the plasma membrane of the basal keratinocyte which has localized condensations called hemidesmosomes, an electron-lucent part, the lamina lucida which contains the sub-basal dense plates and anchoring filaments, the electron-dense lamina densa and the sublamina densa zone or lamina fibroreticularis containing anchoring fibrils.[1–3]

Laminin[4] and type IV collagen[5] which constitute the major proteins of the lamina lucida and lamina densa, respectively, are both widely distributed in tissues. Other BMZ proteins include entactin/nidogen[6,7] and proteoglycans.[8] There are also an increasing number of BMZ proteins which are found only in stratified squamous epithelia. The best characterized is bullous pemphigoid antigen, a 230-kDa glycoprotein[9] which is the target for the disease-associated autoantibodies. A new keratinocyte product-type VII collagen has now been identified as forming anchoring fibrils in the sublamina densa zone of stratified squamous epithelia.[10] A number of antigens have been described within epidermal BMZ from autoantibodies occurring in lichen planus pemphigoides[11] and linear IgA disease,[12] and by fortuitous discovery with monoclonal antibodies, e.g. GB3,[13] AF1 and AF2,[14] and KF1 antibody which has not yet been fully identified.[15]

We describe here a further BMZ antigen localized in the lamina densa. It was detected by a monoclonal antibody (MAb) which had been raised by immunizing mice with human male gonadal germ cells[16] and was previously found to react with sperm tail fibrous sheath.[17] This study therefore deals with the characterization of GDA-J/F3 antigen and its relationship to other known BMZ proteins.

Methods

Specimens

Human tissues (five samples of whole skin, four testes, and single samples from the other tissues) were obtained following surgical removal from organ donors and either snap frozen in liquid nitrogen, or gradually frozen in cold isopentane and then liquid nitrogen. The human foetal tissues (five foetal membranes and three skin) were obtained following the termination of presumed normal pregnancies. Their gestational ages (Table 1) were calculated from standard foetal measurements, including crown–rump length. Foetal membranes (cord, reflected and placental amnion) and a skin sample were also obtained from a single 19-week foetus affected by recessive dystrophic epidermolysis bullosa. This work was undertaken with appropriate ethical committee approval.

Samples were also obtained from skin of inbred BALB/c mice, randomly bred Sprague–Dawley rats, a pig, a cow as well as from skin and gingiva of a macaque monkey.

Human cell lines and cultured keratinocytes

The SVK14 Simian virus 40-transformed keratinocyte[18] and SCC27 squamous cell carcinoma cell lines were maintained in RPMI 1640 medium containing 10% fetal calf serum and added antibiotics. Standard techniques were applied for the isolation and cultivation of keratinocytes from normal adult skin removed from breast reduction specimens.[19]

Monoclonal antibodies

GDA-J/F3 MAb was produced by immunizing BALB/c mice with male gonadal germ cells obtained from an oligozoospermic donor. The details of immunization and screening have been described previously.[16] Anti-type IV collagen antibody was a gift from Dr V.Duance (Bristol). MAB LH7:2[20] to the globular terminal of type VII collagen was used in some comparative studies.

Table 1. Ontogeny of GDA-J/F3 antigen during foetal development

Tissue	EGA*	Reactivity**
Foetal skin	9	+
	12	++
	19	+++
Neonatal skin		+++
Foetal membranes (amnion)	11	−
	15	+
	Term	+++

* EGA, estimated gestational age (weeks).

** +, ++, +++ = Faint, moderate and strong immunostaining respectively.

Indirect immunofluorescence and immunoperoxidase tests of tissues and cells

Frozen sections of the human tissues were studied by routine indirect immunofluorescence (IIF) and immunoperoxidase (IIP) techniques,[16,21] and 1 M NaCl split skin was prepared as previously described.[22] The split skin was then frozen, and cryostat sections were prepared and examined. In the IIF testing of selected frozen sections, the nuclei were counterstained red by incubating the sections with propidium iodide (0·01% in phosphate-buffered saline) for 5 min followed by washing.

Single-cell suspensions of keratinocytes were isolated from normal skin[19] and tested in suspension as for spermatozoa using the standard tube method IIF test.[16,21] The SVK14 and SCC27 cells were subcultured on to sterile slides for 2 days and tested either in a viable state or following overnight air drying with subsequent freezing at −20°C for 2 days.

Immunoelectron microscopy of human tissues with GDA-J/F3 MAb

Both immunogold and immunoperoxidase techniques were used to stain frozen sections of tissue obtained from the human buccal cavity. The 10-μm sections, mounted on to Thermanox cover slips, were incubated with 1:10 diluted GDA-J/F3 MAb overnight at 4°C. Following washing, the samples were incubated with 1:200 diluted biotinylated anti-mouse immunoglobulin antibody (Dakopatt) for 1 h at room temperature. The sections were washed and incubated with either 1:10 diluted gold–streptavidin (15-nm gold particles, Amersham) or 1:50 diluted peroxidase–streptavidin (Dakopatt) for 1 h at room temperature. Following washing, the immunogold-stained sections were ready for processing for electron microscopy while those to be used for immunoperoxidase staining were incubated with diaminobenzidine in hydrogen peroxide and then washed. Sections were then fixed in Karnovsky fixative, post-fixed in osmium tetroxide and processed to resin for sectioning using standard methods.[23]

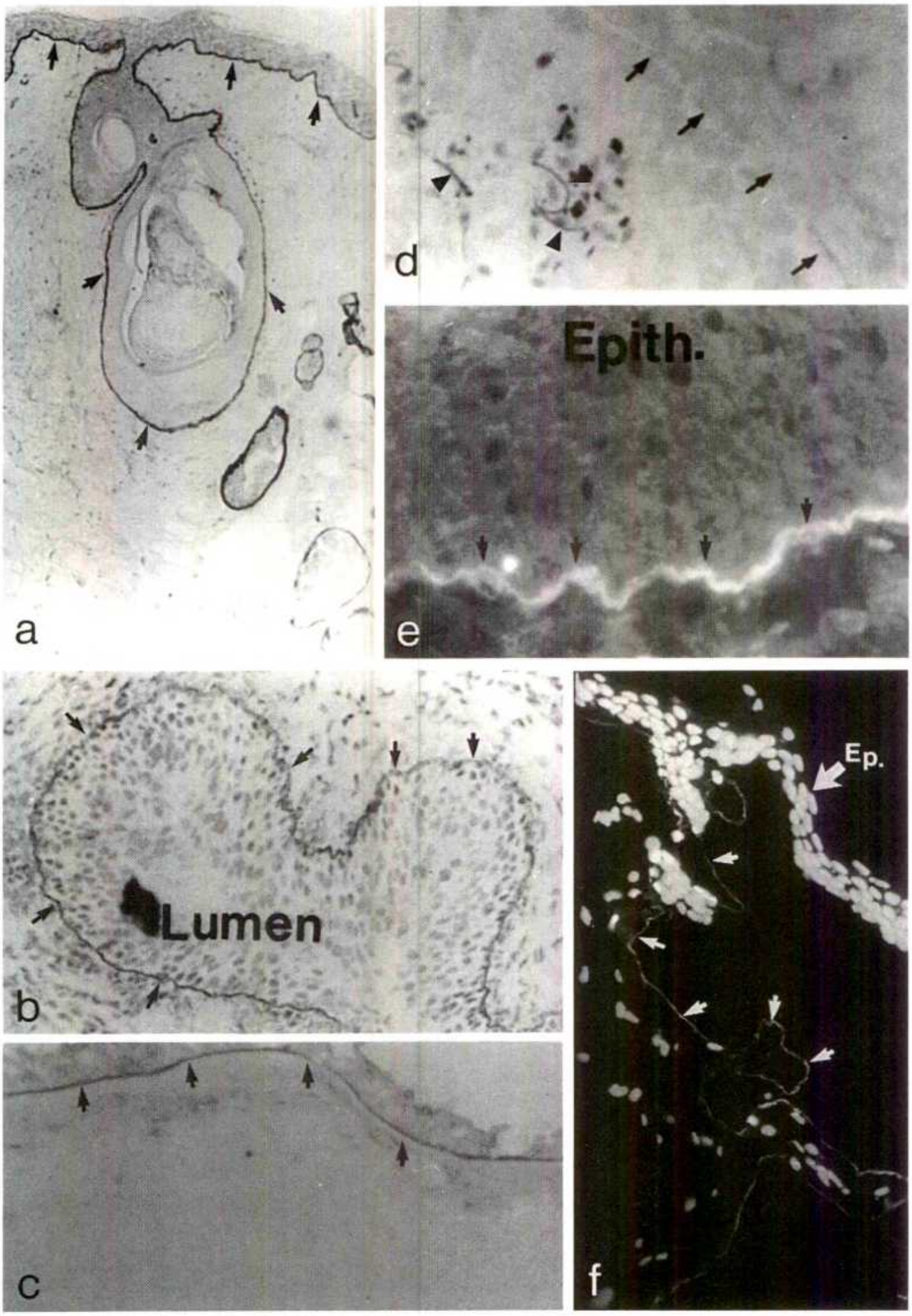

Figure 1. Indirect immunoperoxidase (a–d) and immunofluorescence staining (e,f) of human tissues with GDA-J/F3 MAb. (a), skin, (b) ureter, (c) amnion, (d) testis, and (e) trachea. With the exception of the testis (d) where sperm tails (arrow heads) were stained, the antibody reacted with BMZ (arrows) of all other tissues. The antibody also stained the dermal side (arrows) of split skin (f). Ep, epidermis; epith, epithelium. (a × 120, b × 190, c × 450, d × 490, e × 520, f × 460).

Western blotting of tissue lysates with GDA-J/F3 MAb

Normal skin was split with 1 M NaCl,[22] and the epidermal and dermal fractions were solubilized by overnight incubation in 8 M urea containing 0·3 M mercaptoethanol.[24] In other experiments, the whole skin was solubilized in 1% sodium deoxycholate for 1 h at room temperature. Placental amnion physically separated from chorion was lysed in 8 M urea as described above.

The tissue lysates from all these preparations were electrophoresed through 5%, 7·5% and 12·5% SDS–PAGE gels using reducing and non-reducing conditions.[25] The electrophoresed proteins were transferred on to nitrocellulose,[26] and the unreacted sites were blocked by overnight incubation with PBS containing 0·05% Tween 20 and 20% fetal calf serum. The blotted proteins were tested with the antibodies using a standard immunoperoxidase technique.

Results

Tissue distribution

GDA-J/F3 MAb showed linear staining of the BMZ of all stratified squamous epithelia, including the skin and its appendages (hair follicle and sebaceous gland), toe nail bed, cervix (portio vaginalis), lip, buccal cavity, tonsillar mucous membrane, tongue and oesophagus. The antibody also labelled in a linear fashion the BMZ of some ducts of resting and lactating breasts, amnion, tracheal and nasal ciliated epithelium and ureter (Fig. 1). The antibody did not stain the BMZ of the stomach, ileum, colon, rectum, kidney tubules and glomeruli, liver, lung, fallopian tubes or their blood vessels (data not shown). In the testis, the antibody stained the sperm tails but not the BMZ of the seminiferous tubules (Fig. 1d). Using the IIF technique, the GDA-J/F3 MAb stained the dermal side of split skin (Fig. 1f). The antibody did not react with mouse, rat, pig or cow skin but did stain the BMZ of the monkey skin and gingiva (data not shown).

The reactions of LH7:2 MAb with the BMZ were similar to those of GDA-J/F3 but the LH7:2, unlike GDA-J/F3, failed to react with sperm tails.

Ontogeny of GDA-J/F3 during embryogenesis

Frozen sections of skin and various placental tissues were obtained from foetuses of different gestational ages (Table 1) and tested with GDA-J/F3 MAb using IIF. In a 9-week-old foetus, the antigen was detectable in the skin but not in the chorionic villi or trophoblasts. Similarly the skin but not the amnion or chorionic villi from 12-week-old foetuses showed moderate intensity of fluorescence with GDA-J/F3 MAb. Labelling of amnion with this antibody was first observed at a gestational age of 15 weeks. In a full-term pregnancy, the antigen was detected on the BMZ of reflected, placental and cord amnion but not on other placental tissues (Table 1). Amnion and skin obtained from a foetus affected with generalized recessive dystrophic epidermolysis bullosa were tested with both GDA-J/F3 MAb and LH7:2 by IIF. The BMZ of the amnion and skin failed to react with GDA-J/F3 or LH7:2 (Fig. 2) but did react with the anti-type IV collagen antibody (data not shown).

Reaction of GDA-J/F3 MAb with keratinocytes and cell lines

Using the IIF technique, freshly isolated viable normal skin keratinocytes and the SVK14 and SCC27 squamous cell carcinoma cell lines did not show any surface staining with GDA-J/F3 MAb. Similarly, the antibody did not label the surface or cytoplasm of the non-viable epidermal keratinocytes (cryostat sections of normal skin) or air-dried SVK14 cells. However, the antibody did produce diffuse cytoplasmic staining of the air-dried cultured SCC27 cells with occasional cells showing filamentous immunofluorescence (Fig. 3).

Ultrastructural localization of GDA-J/F3 on BMZ

Sections from human buccal cavity were stained with GDA-J/F3 MAb using the gold and peroxidase immunoelectron microscopy (IEM). In both tests, the localization of GDA-J/F3 to the BMZ was confirmed (Fig. 4). In the peroxidase IEM, the electron-dense area was diffuse but was mainly localized to the lamina densa (Fig. 4a).

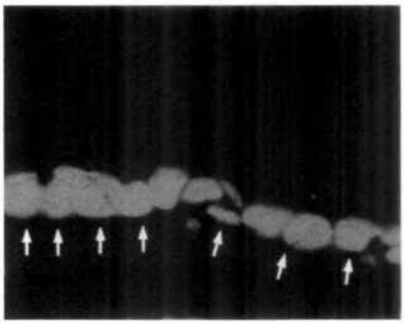

Figure 2. Indirect immunofluorescence staining of placental amnion of RDEB foetus with GDA-J/F3 MAb. The antibody did not stain the BMZ (indicated by arrows). Nuclei were counterstained red with propidium iodide (× 1000).

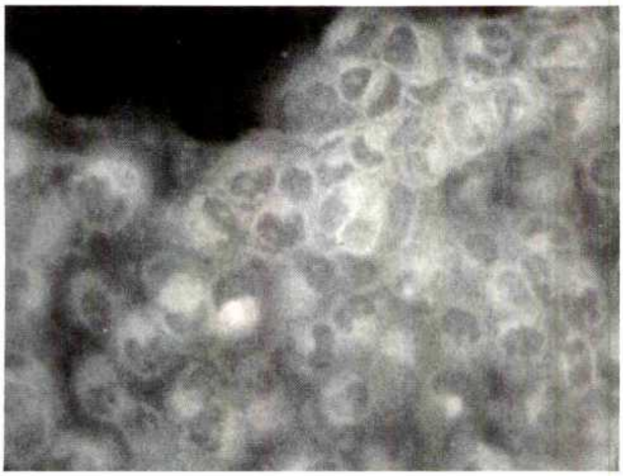

Figure 3. Indirect immunofluorescence staining of SCC27 squamous cell carcinoma cell line with GDA-J/F3 MAb. The antibody produced diffuse cytoplasmic staining (× 280).

However, by using the immunogold, the GDA-J/F3 was more precisely localized to the junction between the lamina densa and sublamina densa zones (Fig. 4b).

Biochemical characterization of GDA-J/F3

Western blotting of tissue lysates with GDA-J/F3 MAb failed to show any specific reaction with proteins derived from dermis, epidermis, whole skin or amnion. LH7:2 which was used as a positive control recognized the 250-kDa type VII collagen in these tissue lysates (data not shown).

Discussion

We have described the use of GDA-J/F3 MAb in the analysis of the BMZ. The antibody was originally raised against male gonadal germ cells[16] and its target antigen localized to the fibrous sheath of the sperm tail.[17] In its reaction with the BMZ, the GDA-J/F3 showed limited tissue distribution to those of stratified squamous epithelia (keratinocytes) and mixed glands such as breast, which have a basal cell of keratinocyte phenotype,[27] ureter (transitional epithelium), trachea and placental amnion.

GDA-J/F3 was localized to the dermal side of split skin and its ultrastructural localization to the lamina densa–sublamina densa region was established by IEM. Based on this localization, it is unlikely that GDA-J/F3 MAb binds lamina lucida BMZ proteins such as the bullous pemphigoid antigen associated with hemidesmosomes,[28] AA3/GB3 antigens[10] and possibly laminin.[4]

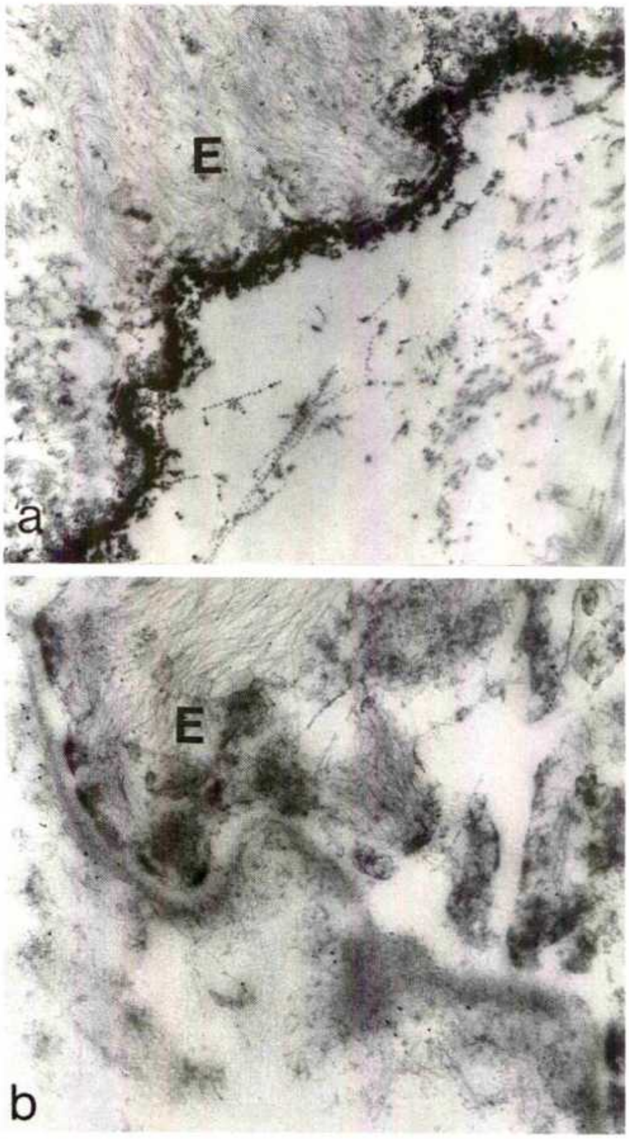

Figure 4. Immunoelectron microscopy of GDA-J/F3 MAb staining of tissues from buccal cavity, (a) immunoperoxidase, (b) immunogold. In (a) the antibody showed diffuse staining mainly localized to the lamina densa. In (b) the gold particles were localized to lamina densa–sublamina densa zone. (a × 26,000, b × 56,000). E, epithelial cell.

Type IV collagen and proteoglycans which are the major components of the lamina densa are also excluded as potential targets for GDA-J/F3 MAb by their ubiquitous tissue distribution in vascular as well as simple epithelial BMZ and also by the difference in the time of appearance during embryogenesis.

A major component of the lamina fibroreticularis of skin and orogenital mucosae (stratified squamous epithelia) is the anchoring fibril, which is made up mainly of type VII collagen which exists in tissue form, as an anti-parallel dimer stretching from lamina densa attachment sites to dermal anchoring plaques.[10] It has now been recognized that a number of monoclonal antibodies, previously described as against BMZ antigens or anchoring fibrils, such as AF1 and AF2,[14] and H3a,[29] react with type VII collagen. MAb LH7:2 recognizes a 60-kDa polypeptide from the globular terminal region of type VII collagen and, with post-embedding labelling, its target antigen has been localized to the junction of the lamina densa and dermis;[30] and, with pre-embedding technique, to anchoring fibrils and anchoring plaques,[31] a locality which is similar to that of GDA-J/F3. It is therefore possible that GDA-J/F3 MAb recognizes an antigen which may be associated with type VII collagen. This is further supported by the similarity in the tissue distribution of both GDA-J/F3 and LH7:2 antigens,[32] with the exception that GDA-J/F3 unlike LH7:2, is also expressed in the sperm tail.

Further evidence was obtained from studies of skin from patients with generalized recessive dystrophic epidermolysis bullosa (RDEB). In a single case of generalized RDEB, now shown by many investigators to involve type VII collagen,[20,33,34] both GDA-J/F3 and LH7:2 were lacking.[35] In RDEB, anchoring fibrils are absent or abnormal[36] and type VII collagen antigens are lost or diminished in many cases.[20,34] However, other mechanisms are thought to be involved in the pathogenesis of RDEB and the disease may represent a mixed bag of genetic abnormalities, which could include mutations in the type VII collagen itself, a defect in its extracellular transfer,[37,38] changes in collagen receptors or binding proteins, and altered collagenases or metalloproteinases involved in collagen degradation. An altered collagenase has been reported in RDEB fibroblasts.[39] Consequently although GDA-J/F3 could detect type VII collagen, the possibility that it could be a collagen receptor/binding protein or a collagenase inhibitor (loss of which might enhance collagenase activity) cannot be ruled out. GDA-J/F3 is likely to be an epithelial cell product because the antigen is detectable in the SCC27 cell line which is of keratinocyte origin. Type VII collagen, which could be synthesized by both keratinocytes and fibroblasts[40,41] has been reported missing from supernatant and cell extracts of RDEB fibroblasts.[42]

Biochemically, both the BMZ and fibrous sheath of sperm tails are difficult to solubilize, and the extraction of their antigens often requires the use of urea.[17] In Western blotting, however, even urea extracts of fibrous sheath,[17] dermis, epidermis and whole skin as well as amnion failed to react with GDA-J/F3 MAb. This could be due to denaturation of the antigenic site by urea, or the antibody may recognize a conformational epitope.[17] Whether the reaction of GDA-J/F3 MAb with both skin and fibrous sheath was due to the presence of the same protein or just an epitope needs to be elucidated. Screening of GDA-J/F3 MAb with spermatozoa from RDEB patients (whose skin lacks the antigen) may shed some light on the relationship and genetic control of the antigen(s) in both the skin and sperm. The spermatozoa from the paternal obligate heterozygote might also be of interest being haploid in type.

Human autoantibodies to the globular terminal of type VII collagen appear in the disease epidermolysis bullosa acquisita[24,43], and react with guinea-pig, rabbit and human tissues.[44] LH7:2 and GDA-J/F3 MAbs do not cross react with other species (apart from monkeys) reflecting differences in sensitivity of polyclonal antisera and MAbs, and possible subtle sequence divergence in the corresponding determinants. The constant association of GDA-J/F3 with cells of keratinocyte phenotype marked by the expression of basal keratins 5 and 14[27] suggests a role in keratinocyte-specific function, possibly adhesion or mediation of epithelial–mesenchymal interactions.

Note added in proof

Recent *en bloc* labelling for IEM has shown localization of GDA-J/F3 to the lateral border of the proximal banded region of the anchoring fibril.

Acknowledgments

We are indebted to D.Auger (Oral Pathology) for his help with the electron microscopy, and the Leverthulme Trust for their financial support.

References

1 Katz SI. The epidermal basement membrane zone: Structure, ontogeny and role in disease. *J Am Acad Dermatol* 1984; 11: 1025–37.

2 Briggaman RA. Biochemical composition of the epidermal–dermal junction and other basement membrane. *J Invest Dermatol* 1982; 78: 1–6.

3 Eady RAJ. The basement membrane. Interface between the epithelium and the dermis: structural features *Arch Dermatol* 1988; 124: 709–12.

4 Timple R, Rhode H, Gehron Robey P *et al.* Laminin: a glycoprotein from basement membrane. *J Biol Chem* 1979, 254: 9933–7.

5 Yaoita H, Foidart JM, Katz SI Localisation of the collagenous component in skin basement membrane. *J Invest Dermatol* 1978; 70: 191–3.

6 Carlin B, Jaffe R, Bender B, Chung AE Entactin, a novel basal lamina associated sulphated glycoprotein *J Biol Chem* 1981; 256: 5209–14.

7 Timple R, Dziadek M, Fujiwora S *et al.* Nidogen: A new self-

aggregating basement membrane protein. *Eur J Biochem* 1983; **137:** 455–65.

8 Fine J-D, Horiguchi Y, Jester J, Couchman JR. Detection and partial characterisation of a mid lamina lucida-hemidesmosome-associated antigen (19-DEJ-1) present within human skin. *J Invest Dermatol* 1989; **92:** 825–30.

9 Stanley JR, Woodley DT, Katz SI. Identification and characterisation of pemphigoid antigen extracted from normal human skin. *J Invest Dermatol* 1984; **82:** 108–11.

10 Keene DR, Sakai LY, Lunstrum GP *et al.* Type VII collagen forms an extended network of anchoring fibrils. *J Cell Biol* 1986; **104:** 818–26.

11 Davies AL, Bhogal B, Whitehead PC *et al.* Lichen planus pemphigoides: Its relationship to bullous pemphigoid. *J Invest Dermatol* 1991; (in press).

12 Wojnarowska F, Whitehead P, Leigh IM *et al.* Identification of the target antigen in chronic bullous disease of childhood and linear IgA disease in adults. *J Invest Dermatol* 1991; (in press).

13 Verrando P, Hsi B, Yeh C *et al.* Monoclonal antibody GB3, a new probe for the study of human basement membranes and hemidesmosomes. *Expt Cell Res* 1987; **170:** 116–28.

14 Goldsmith LA, Briggaman RA. Monoclonal antibodies to anchoring fibrils for the diagnosis of epidermolysis bullosa. *J Invest Dermatol* 1983; **81:** 464–6.

15 Bernard BA. Biochemical characterisation of the epithelial membrane antigen defined by the monoclonal antibody KF-1. *J Invest Dermatol* 1986; **87:** 86–8.

16 Jassim A, Festenstein H. Molecular dissection of human testicular germ cell differentiation with monoclonal antibodies. *J Reprod Immunol* 1987; **12:** 173–89.

17 Jassim A, Auger D, Oliver RTD, Sachs J. GDA-J/F3 monoclonal antibody as a novel probe for the human sperm tail fibrous sheath and its anomalies. *Hum Reprod* (1990); **5:** 990–6.

18 Taylor-Papadimitriou J, Purkis P, Lane EB *et al.* Effects of SV40 transformation on the cytoskeleton and behavioural properties of human keratinocytes. *Cell Differ* 1982; **11:** 169–80.

19 Leigh IM, Purkis PE, Navsaria HA, Phillips TJ. Treatment of chronic venous ulcers with sheets of cultured allogenic keratinocytes. *Br J Dermatol* 1987; **117:** 591–7.

20 Leigh IM, Eady RAJ, Heagarty AHM *et al.* Type VII collagen is a normal component of epidermal basement membrane which shows altered expression in recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 1988; **90:** 639–42.

21 Jassim A, Festenstein H. Immunological and morphological characterisation of nucleated cells other than sperm in semen of oligospermic donors. *J Reprod Immunol* 1987; **11:** 77–89.

22 Gammon WR, Briggaman RA, Inman AO *et al.* Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by direct immunofluorescence on 1·0 M sodium chloride-separated skin. *J Invest Dermatol* 1984; **82:** 139–44.

23 Bowdler AL, Griffiths DFR, Newman GR. The morphological and immunohistochemical analysis of renal biopsies by light and electron microscopy using a single processing method. *Histochem J* 1989; **21:** 393–402.

24 Tatnall FM, Whitehead PC, Black MM *et al.* Identification of the epidermolysis bullosa acquisita antigen by LH 7.2 monoclonal antibody: use in diagnosis. *Br J Dermatol* 1989; **120:** 533–9.

25 Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 1970; **227:** 680–5.

26 Towbin HT, Straehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels onto nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76:** 4350–4.

27 Purkis PE, Steel JB, MacKenzie IE *et al.* Antibody markers of basal cells in complex epithelia: *J Cell Sci* 1990; **97:** 39–50.

28 Stanley JR, Hawley-Nelson P, Yuspa SH *et al.* Characterisation of bullous pemphigoid antigen—a unique basement membrane protein of stratified squamous epithelia. *Cell* 1981; **24:** 897–903.

29 Paller AS, Queen LL, Woodley DT *et al.* A mouse monoclonal antibody against a newly discovered basement membrane component, the epidermolysis bullosa acquisita antigen. *J Invest Dermatol* 1985; **84:** 215–17.

30 Tidman MJ, Eady RAJ. Evaluation of anchoring fibrils and other components of dermal–epidermal junction in dystrophic epidermolysis bullosa by a quantitative ultrastructural technique. *J Invest Dermatol* 1985; **84:** 374–99.

31 Shimizu H, McDonald JN, Gunner DB *et al.* Epidermolysis bullosa acquisita and the carboxy terminus of type VII collagen have a common immunolocalization to anchoring fibrils and lamina densa of basement membrane. *B J Dermatol* 1990; **122:** 577–85.

32 Leigh IM, Purkis PE, Bruckner-Tuderman L. LH 7.2 monoclonal antibody detects type VII collagen in the sub-lamina densa zone of ectodermally-derived epithelia, including skin. *Epithelia* 1987; **1:** 17–29.

33 Heagarty AHM, Kennedy AR, Leigh IM *et al.* Identification of an epidermal basement defect in recessive forms of dystrophic epidermolysis bullosa by LH 7:2 monoclonal antibody: use in diagnosis. *Br J Dermatol* 1986; **115:** 125–31.

34 Brucker-Tuderman L, Ruegger S, Odermatt B *et al.* Lack of type VII collagen in unaffected skin of patients with severe recessive dystrophic epidermolysis bullosa. *Dermatologica* 1988; **176:** 57–64.

35 Heagerty AHM, Tidman MJ, Leigh IM *et al.* Dystrophic epidermolysis bullosa: monoclonal antibodies reveal heterogeneity within clinical groups. *Br J Dermatol* 1986; **115** (Suppl. 30): 20.

36 Briggaman RA, Wheeler CE. Epidermolysis bullosa dystrophica-recessive: a possible role of anchoring fibrils in the pathogenesis. *J Invest Dermatol* (1975); **65:** 203–11.

37 Smith LT, Sybert VP. Intraepidermal retention of type VII collagen in a patient with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 1990; **94:** 261–4.

38 Fine JD, Horiguchi Y, Stein DH *et al.* Intra epidermal type VII collagen, evidence for abnormal intracytoplasmic processing of a major basement membrane protein in rare patients with dominant and possible localized recessive forms of dystrophic epidmolysis bullosa. *J Am Acad Dermatol* 1990; **221:** 188–95.

39 Bauer EA, Eisen AZ. Recessive dystrophic epidermolysis bullosa: evidence for increased collagenase as a genetic characteristic in cell culture. *J Exp Med* 1978; **148:** 1378–87.

40 Stanley JR, Rubenstein N, Klaus-Kovtun V. Epidermolysis bullosa acquisita antigen is synthesized by both human keratinocytes and human dermal fibroblasts. *J Invest Dermatol* 1985; **85:** 542–5.

41 Bruckner-Tuderman L, Schnyder UW, Winterhater KH, Bruckner P. Tissue form of type VII collagen from human skin and dermal fibroblasts in culture. *Eur J Biochem* 1987; **165:** 607–11.

42 Bruckner-Tuderman L, Mitsuhashi Y, Schnyder UW, Buckner P. Anchoring fibrils and type VII collagen are absent from skin in severe recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 1989; **93:** 3–9.

43 Woodley DT, Burgeson RE, Lunstrum G *et al.* Epidermolysis bullosa acquisita antigen is the globular carboxyl terminus of type VII procollagen. *J Clin Invest* 1988; **81:** 683–7.

44 Furue M, Iwata M, Tamaki K, Ishibashi Y. Anatomical distribution and immunological characteristics of epidermolysis bullosa acquisita antigen and bullous pemphigoid antigen. *Br J Dermatol* 1986; **114:** 651–9.

British Journal of Dermatology (1991) 125, 108–115. ADONIS 000709639100179B

Interleukin-5-induced granulocyte activation in atopic patients

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Accepted for publication 30 January 1991

Summary

The effect of recombinant human and murine interleukin-5 (IL-5) on granulocytes was investigated in patients with atopic dermatitis (AD) and allergic rhinitis and compared with those from patients with plaque psoriasis and normal non-atopic controls. Granulocyte activation was measured as lucigenin-dependent chemiluminescence (CL) and release of eosinophil cationic protein (ECP) as well as by scanning- and transmission-electron microscopy (EM) and the ultrastructural detection of production of H_2O_2. A significant direct effect of both human and murine IL-5 on granulocyte oxidative metabolism could only be detected in those patients with AD and allergic rhinitis. Also, as compared to normal controls, significantly increased CL responses were observed in these patients with stimulation with other granulocyte-activating cytokines, particularly GM-CSF. In patients with psoriasis there was no significant increase in response to stimulation with TNFα, TNFβ or GM-CSF, but IL-5 induced slight but significant CL responses in the granulocytes. None of the cytokines tested significantly stimulated the release of ECP in any of the groups.

Ultrastructural studies showed that stimulation with human as well as murine IL-5 produced significant morphological changes in both eosinophils and polymorphonuclear neutrophilic granulocytes from the patients with AD. Production of H_2O_2 was visualized at the luminal part of the intracytoplasmic vesicles of the granulocytes and at the points of contact between the cells. On morphometric analysis, almost all the polymorphonuclear neutrophils in the patients with AD appeared to be activated, whereas only some of these cells in the normal controls showed signs of activation.

Interleukin 5 (IL-5) is a T-lymphocyte-derived cytokine that was first described in the mouse as an eosinophil differentiation factor (EDF), able to stimulate the production of eosinophils in long-term cultures of bone marrow.[1] Murine EDF/IL-5 also stimulated various activities of human eosinophils.[2] This factor was found to be identical with B-cell growth factor II,[3] but murine EDF/IL-5 had no effect on human B cells.[4]

The gene encoding human IL-5 was cloned revealing that human IL-5 had no significant homology with other known regulators of haemopoietic growth.[5,6] The amino-acid sequence, however, showed a 70% homology with that of the murine EDF/IL-5.[6] As with the mouse factor, human IL-5 stimulated the production of eosinophils and eosinophil colonies from normal human bone marrow, but had no effect on the production of neutrophils or mononuclear cells,[6] nor did it affect human B cells.[4]

Recombinant human IL-5 was shown to be a selective activator of human eosinophil function.[7] Stimulation of eosinophils induced significant morphological changes, leading to a polarization of the cells. IL-5 increased the capacity of human blood eosinophils to kill antibody-coated tumour cells, enhanced phagocytosis of opsonized yeast cells and was shown to be a selective eosinophil chemoattractant.[7,8] Furthermore, recombinant murine IL-5 was found to enhance the viability of eosinophils and to mediate their conversion to the hypodense phenotype.[9]

Recent studies provide evidence for the potential role of a subset of $CD4^+$ T lymphocytes in the pathogenesis of atopic disease.[10] These T cells are very similar to the Th2 cell subtype described in the murine system which preferentially produces the eosinophil-activating cytokine IL-5, as well as IL-4 and IL-6, whereas Th1 cells specifically release IL-2, IFN-γ, and TNFβ.[11] T-cell clones, specific for house dust mite *Dermatophagoides pteronissinus*, were generated from the peripheral blood of atopic patients and a non-atopic individual.[12] Only antigen-

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specific T-cell clones derived from the atopic donors showed a cytokine secretion profile very similar to the murine Th2 subtype, supporting the possible role of this T-cell subset for the pathogenesis of atopic disease. Activated eosinophils and, in part, polymorphonuclear neutrophilic granulocytes (PMN) play an important role in the propagation of allergic inflammation and are capable of mediating tissue damage, particularly by the release of reactive oxygen species and granule contents.[13,14] To examine the potential importance of IL-5 in the pathogenesis of atopic diseases, the direct effect of this cytokine on granulocytes from patients with atopic dermatitis, allergic rhinitis and, as controls, patients with psoriasis and healthy blood donors was assessed by both functional and ultrastructural assay systems.

Methods

Subjects

The following groups of patients and controls were tested.

1. Five patients with perennial and seasonal allergic rhinitis as estimated by personal history, skin prick tests and laboratory findings. Mean log IgE ± SEM was 1·9682 ± 0·0655 kU/l, geometric mean 92·6 kU/l.
2. Five patients with atopic dermatitis with moderate to severe disease activity: ≥ 25% of body surface area was involved. The diagnosis was established according to the criteria of Hanifin and Rajka.[15] Mean log IgE ± SEM was 3·2753 ± 0·2155 kU/l, geometric mean 1885·3 kU/l.
3. Five patients with psoriasis with extensive involvement: ≥ 25% of body surface area involved. Mean log IgE ± SEM was 1·3035 ± 0·2630 kU/l, geometric mean 20·1 kU/l.
4. Five non-atopic healthy blood donors. Mean log IgE was 1·3097 ± 0·2892 kU/l, geometric mean 20·4 kU/l.

Atopy was excluded in the patients with psoriasis and normal controls by history and laboratory findings (measurement of total IgE and specific IgE to timothy grass, cultivated rye grass, common silver birch, mugwort, cat, *Cladosporium herbarum*, *D. pteronissinus*). Neither the controls nor the patients had received any medication for at least 3 weeks prior to collection of the blood specimens.

Cytokines

Recombinant human IL-5 (specific activity 10^6U/mg protein) was obtained from Amgen, CA, U.S.A. Recombinant murine IL-5 was purchased from Genzyme, Boston, MA, U.S.A. The recombinant human tumour necrosis factor α (TNFα) and TNFβ were gifts from BASF, Ludwigshafen, Germany, with a specific activity of 8.5×10^6 U/mg and 6×10^7 U/mg protein for TNFα and TNFβ, respectively. Recombinant human GM-CSF, with a specific activity of $1{\cdot}1 \times 10^8$ U/mg in the CML assay, was a gift from Glaxo Research, Geneva, Switzerland. Before testing, all cytokines were diluted in phosphate-buffered saline containing 50 μg/ml bovine serum albumin (BSA).

Reagents and antibodies

Ficoll–Paque solution was obtained from Pharmacia (Freiburg, Germany); purified BSA and lucigenin were obtained from Sigma (Munich, Germany). Culture media and antibiotics were purchased from Biochrom-Seromed (Berlin, Germany). $CeCl_3$ and 3-amino-1,2,4 triazole (AT) were from Fluka, Buchs, Switzerland. Reagents and media for electron microscopy were obtained from Roth, Karlsruhe, Germany.

Isolation of human granulocytes

Human granulocytes were isolated from heparinized venous blood from healthy blood donors, as previously described,[16] by Ficoll gradient centrifugation and three 30-s cycles of treatment with 0·2% NaCl followed by the addition of an equal volume of 1·6% NaCl to lyse the red blood cells. The resulting granulocyte preparations from non-atopic controls contained, in addition to PMN, 4·8 ± 0·5% eosinophilic granulocytes as judged by Pappenheim staining of the cytospin preparations (Fig. 1). Cells were more than 95% viable as estimated by trypan blue. Subsequently, granulocytes were suspended in different media as indicated below.

Lucigenin-dependent chemiluminescence

Lucigenin-dependent chemiluminescence (CL)[16,17] in contrast to the luminol-enhanced reaction is independent of the release of peroxidase[18] and represents an extremely sensitive measure of the release of reactive oxygen species (ROS) from granulocytes. However, the CL response is not known to be strongly correlated with the production of a particular ROS. For CL measure-

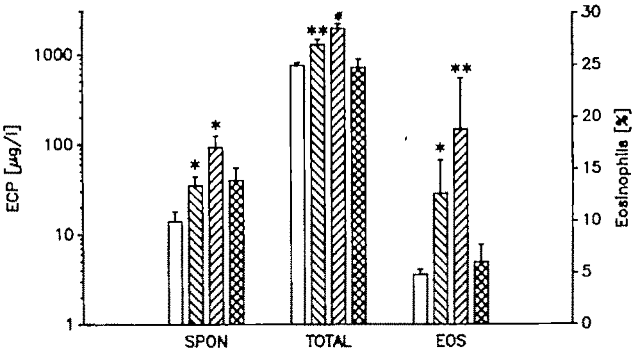

Figure 1. Eosinophil counts (EOS), ECP content (TOTAL) and spontaneous release (SPON) of ECP in granulocyte preparations of patients and controls. Values represent the mean ± SEM of experiments on five patients or controls. * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$. □, controls; ■, inhalant allergy; ■, atopic dermatitis; ■, psoriasis.

ments, granulocytes were subsequently suspended to a density of 5×10^6 cells/ml in HEPES-buffered Hank's balanced salt solution (HBSS), pH 7·4, containing 2 mM lucigenin and 1 mg/ml BSA. Aliquots (200 μl), containing 1×10^6 cells each, were placed into unsealed polystyrene luminescence tubes (Lumacuvette/Lumac, Landgraaf, the Netherlands) and stored at 4°C for a maximum of 6 h prior to use. Measurements were performed at 37°C in two six-channel Biolumat LB 9505C (Berthold, Wildbad, Germany) interfaced via Siemens PCD-2 computers to Epson EX800 colour graphic printers. Fifty microlitres of the stimulus were added to the granulocytes corresponding to an 1:5 dilution. Integral counts from a 0–60-min incubation interval after addition of stimuli to the granulocytes were measured.

Release of eosinophil cationic protein

Granulocytes were suspended at a concentration of 5×10^6 cells/ml in PBS, containing 1 mM Ca^{2+} and 0·5 mM Mg^{2+}. Assays were performed in 96-well flat bottom tissue-culture plates in the presence of 5 μg/ml cytochalasin B using 5×10^5 cells/well. After addition of the stimulus granulocytes were incubated for 60 min at 37°C, slightly shaken on a Titertek microplate shaker and centrifuged immediately. Total cellular eosinophil cationic protein (ECP) activity was determined by addition of CETAB to the granulocytes at a final concentration of 0·4%. ECP was detected in the supernatants using the RIA technique (ECP RIA, Pharmacia-LKB, Freiburg, Germany). Results were expressed as per cent of total content.

Ultrastructural detection of granulocyte activation

Ultrastructural detection of granulocyte activation was performed as previously described.[19,20] In brief, granulocytes were incubated at a concentration of 5×10^6/ml in microtubes and at 1×10^6/ml in Leighton tubes (Costar, Fernwald, Germany) for transmission and scanning electron microscopy (EM), respectively. Thereafter, stimuli or medium were added to the cells for 15 min at 37°C. After the incubation period granulocytes were fixed immediately for transmission or scanning EM as previously described,[20] or they were subjected to hydrogen peroxide detection and subsequently fixed for transmission EM.

For detection of hydrogen peroxide production[19,20] unfixed cells were pre-incubated following centrifugation for 10 min at 37°C in 0·1 M Tris-maleate with 7% sucrose, pH 7·5, containing 1 mM AT. Granulocytes were then incubated for 30 min at 37°C in 0·1 M Tris-maleate with 7% sucrose, pH 7·5, 10 mM AT and 1 mM $CeCl_3$. Finally, the cells were briefly washed in Tris-maleate buffer with 7% sucrose at 4°C and fixed in 2% glutaraldehyde in 0·1 M sodium cacodylate buffer, pH 7·3, with 5% sucrose for 60 min at 4°C. In the cerium precipitation series cells were subsequently washed in sodium cacodylate buffer, pH 6·0, with 5% sucrose for 60 min at 4°C.

Morphometric analysis of granulocyte activation

The quantity of activated granulocytes was calculated by means of scanning EM. Six random-sampled high-power fields of the Leighton tube culture area were evaluated and 100–200 cells per field were counted. Rounded cells with ruffled surface membranes were termed as 'unstimulated'. Cells which were flattened and typically polarized were defined as 'activated'. The results are given in per cent activated cells.

Measurement of total IgE and specific IgE

All measurements were performed using the Pharmacia CAP system by IgE FEIA and RAST FEIA (Pharmacia-LKB, Freiburg, Germany).

Statistical analysis

Statistical evaluations were performed by using Mann–Whitney *U*-test. For evaluation of correlations, Spearman's rank correlation coefficient was calculated and tested for statistical significance.

Results

IL-5-induced CL response in granulocytes of patients and controls

In contrast to normal non-atopic subjects the granulocytes of patients with atopic dermatitis (AD) responded to stimulation with human IL-5 in a dose-dependent fashion in the range of 1–10 U/ml. Higher concentrations of IL-5 did not increase the rate of ROS production (Fig. 2a). Similar effects were obtained when murine IL-5 was used in the range of 10–1000 U/ml (Fig. 2b). The CL response induced by human as well as murine IL-5 showed a typical time course starting after a lag phase of 3–5 min after addition of the stimulus, with a peak at about 10–15 min followed by a long-lasting increase of baseline activation of the granulocytes. At low concentrations of IL-5 (1 U/ml) the lag phase and peak shifted in time (25–30 min and 40–50 min, respectively) (Fig. 3).

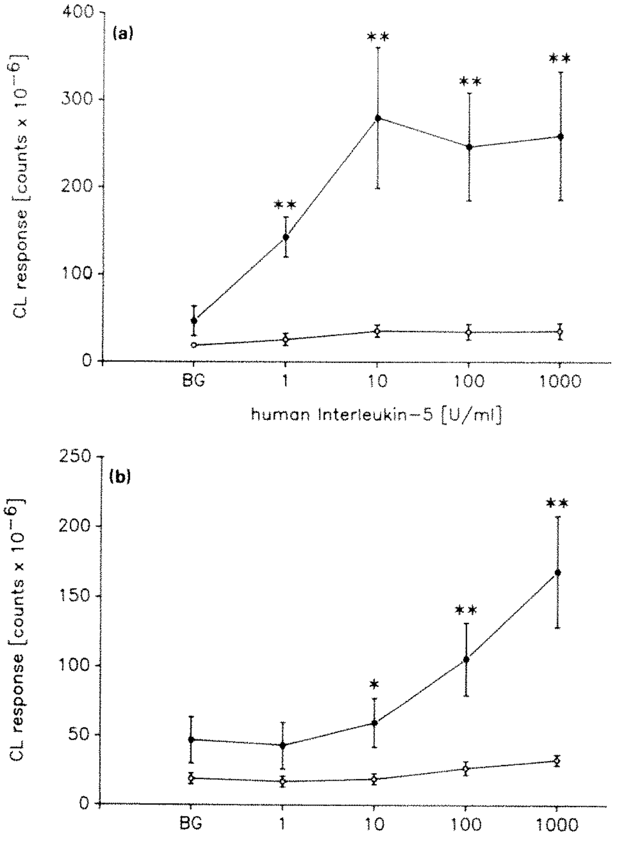

Figure 2. IL-5-induced chemiluminescence response of granulocytes from patients with atopic dermatitis and normal controls: effect of recombinant (a) human and (b) murine IL-5. Integral counts were measured for 60 min. BG, background, induced by addition of medium alone. Values represent the mean ± SEM of experiments on five patients or controls. ** $P \leq 0{\cdot}01$. ●——●, atopic dermatitis; ○——○, controls.

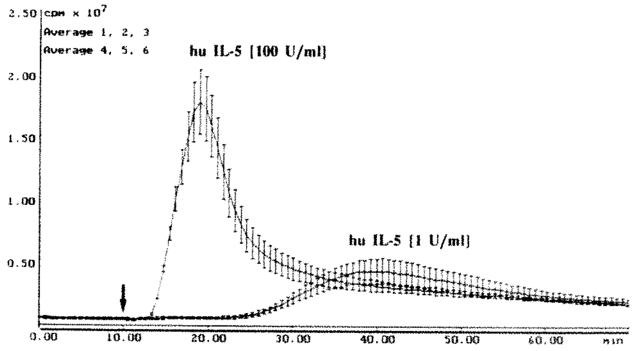

Figure 3. Representative time course of the CL response induced by recombinant human IL-5 in AD. Arrow indicates addition of the stimulus. Values represent the mean ± SE of triplicate measurements on a single AD patient.

In order to examine the specificity of the IL-5-induced effect on granulocytes from AD patients, patients with allergic rhinitis, as an example of an additional atopic disease, and psoriatic patients, as an example of an inflammatory skin disorder with 'non-allergic' pathogenesis, were tested. Significant CL responses of granulocytes upon stimulation with IL-5 were seen in patients with allergic rhinitis (Fig. 4). In contrast, IL-5 induced a slight, but statistically significant, CL response in granulocytes of patients with psoriasis (Fig. 4). However, the effects were significantly lower compared to patients with AD or allergic rhinitis.

To exclude the possibility that increased CL responses are simply due to increased numbers of eosinophils in granulocyte preparations of patients with AD and

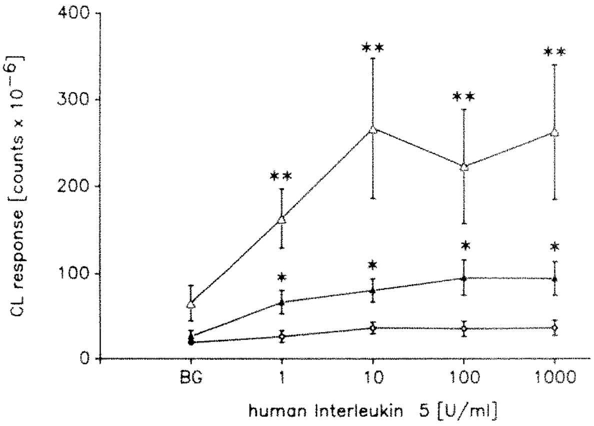

Figure 4. Chemiluminescence response of granulocytes from patients with inhalant allergy, psoriasis and normal controls upon stimulation with recombinant human IL-5. Integral counts were measured for 60 min. BG, background, induced by addition of medium alone. Values represent the mean ± SEM of experiments on five patients or controls. * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$. △——△, inhalant allergy; ▲——▲, psoriasis; ○——○, controls.

allergic rhinitis, the results were calculated as CL response/5 × 10^4 eosinophils. Qualitatively similar results were obtained (data not shown).

CL response in granulocytes of patients and controls upon stimulation with GM-CSF, TNFα and TNFβ

Long-lasting production of reactive oxygen species in human granulocytes can only be induced by selected cytokines, including TNFα, TNFβ and GM-CSF.[13,19,21] To examine the specificity of the IL-5-inducing granulocyte activation in atopic disease, the effect of TNFα and β (10^3U/ml), and GM-CSF (10^3U/ml) on granulocytes was evaluated. Significantly increased CL responses were observed upon stimulation with GM-CSF and to a lower degree with TNFβ in patients with both AD and allergic rhinitis (Fig. 5). In addition, CL responses upon stimulation with TNFα were clearly enhanced in AD patients (Fig. 5). In patients with psoriasis no significantly increased responses were detected upon stimulation with TNFα, TNFβ or GM-CSF (Fig. 5).

Effect of IL-5 on the release of eosinophil cationic protein (ECP) by eosinophils of patients and controls

The granulocyte preparations of patients with AD or allergic rhinitis contained an increased number of eosinophils (Fig. 1) and an increased content of ECP contained in the granules.[20] There was also a significant correlation between the eosinophil count and ECP in the granulocyte preparations ($R = 0{\cdot}684$, $P \leq 0{\cdot}005$). Associated with the increased numbers of eosinophils there was an increased spontaneous release of ECP in the supernatant in patients with allergic rhinitis and AD (Fig. 1).

To standardize for differences in eosinophil count, release of ECP was calculated as percentage of total content. Granulocytes were stimulated with human as well as murine IL-5 in a range of 1–1000 U/ml and the effects of TNFα and β (10^3U/ml), and GM-CSF (10^3U/ml) were also tested. None of these stimuli enhanced the release of ECP by granulocytes from any of the groups tested (data not shown).

Morphometric analysis of IL-5-induced granulocyte activation

To determine whether PMN, eosinophils or both cell types are the cellular source of ROS production, the effect of IL-5 on granulocytes from patients with AD was evaluated by morphometric analysis using scanning EM (Fig. 6). It was shown that upon stimulation with IL-5 (10^2U/ml), 85 ± 5% of the patients' cells showed morphological signs of activation in contrast to 30 ± 1% for normal controls (Figs 6 and 7).

IL-5-induced ultrastructural changes and localization of hydrogen peroxide production

On stimulation with IL-5 most of the granulocytes of patients with AD showed characteristic morphological changes within 15 min (Fig. 7). The granulocytes were

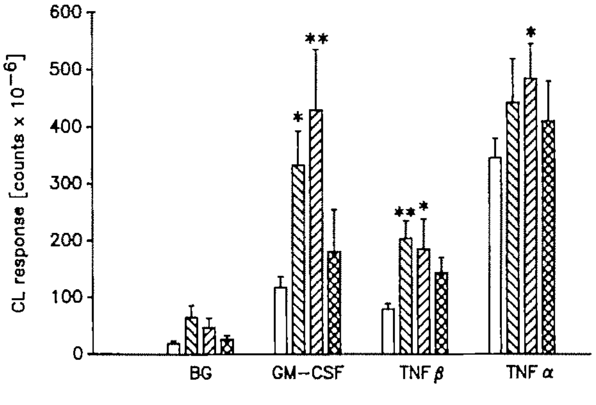

Figure 5. Chemiluminescence response of granulocytes from patients with atopic dermatitis, inhalant allergy, psoriasis and normal controls: effect of recombinant human GM-CSF (10^3U/ml), TNFα (10^3U/ml) and TNFβ (10^3U/ml) Integral counts were measured for 60 min. BG: background, induced by addition of medium alone. Values represent the mean ± SEM of experiments on five patients or controls * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$. □, controls; ■, inhalant allergy; ▨, atopic dermatitis; ▩, psoriasis.

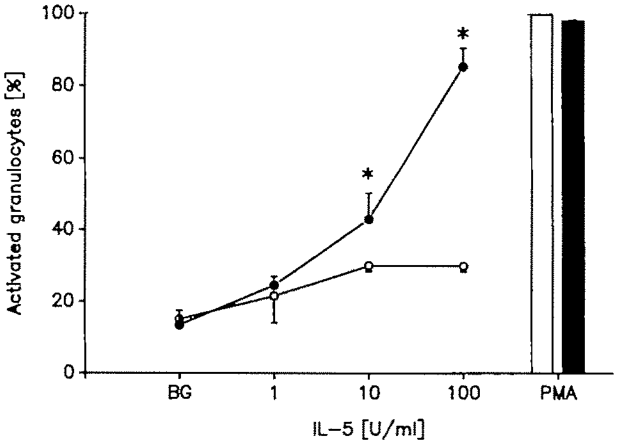

Figure 6. Morphometric analysis of granulocyte activation upon stimulation with recombinant human IL-5 based on scanning EM in patients with AD and controls. PMA (10 ng/ml) served as a control stimulus. Results represent the mean ± SEM of separate experiments on three patients or controls. * $P \leq 0{\cdot}05$. ●——●, atopic dermatitis; ○——○, controls.

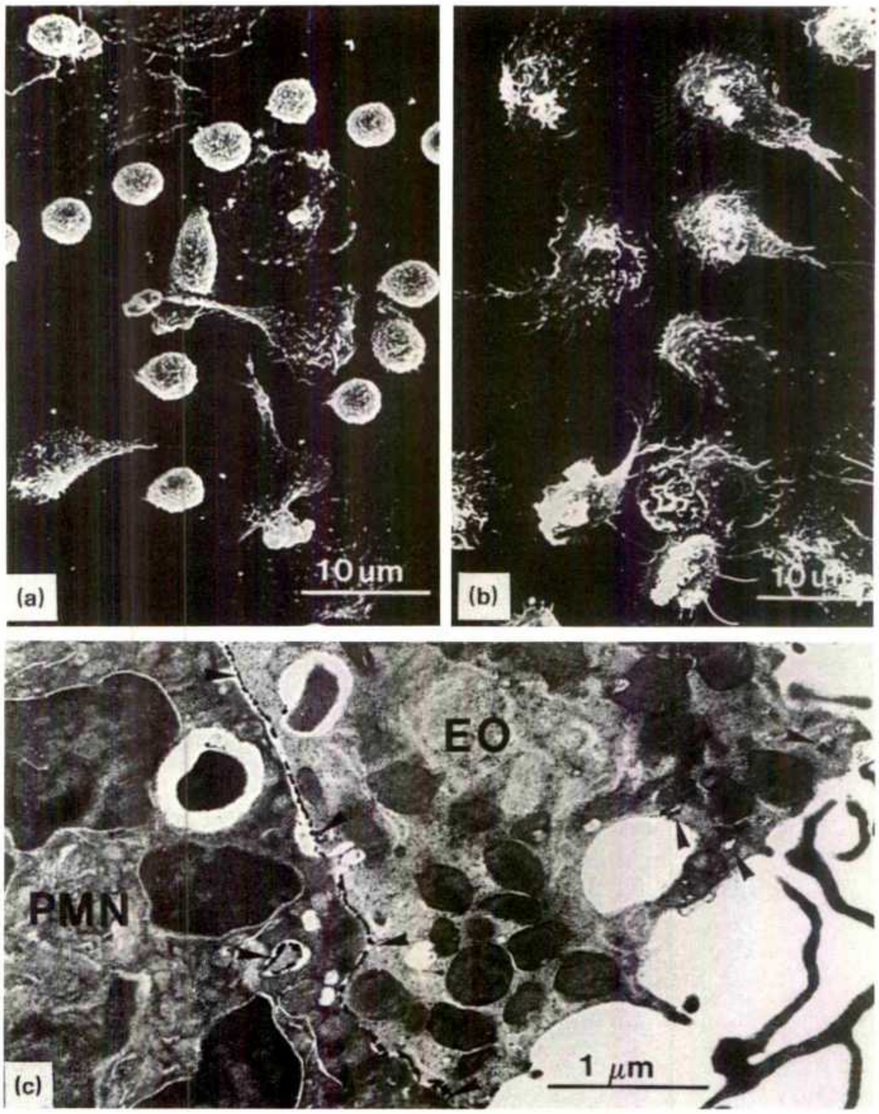

Figure 7. Ultrastructural detection of granulocyte activation induced by recombinant human IL-5. (a) Granulocytes of a normal non-atopic control stimulated with IL-5 (100 U/ml), 15 min at 37°C, scanning EM; (b) granulocytes of a patient with AD stimulated with IL-5 (100 U/ml), 15 min at 37°C, scanning EM; (c) ultrastructural detection of hydrogen peroxide production in granulocytes of a patient with AD stimulated with IL-5 (100 U/ml), 15 min at 37°C, transmission EM; the reaction product is seen in the contact zone between adjacent PMN and eosinophils as well as in intracytoplasmic vacuoles of PMN and eosinophils (arrows).

flattened and adhered to the plastic layer, developing pseudopodia-like protrusions at one side of the cell (Fig. 7b). In contrast the unstimulated cells remained rounded with ruffled surface membranes (Fig. 7a).

Hydrogen peroxide, produced during activation of the granulocytes, was visualized by electron microscopy when the electron-dense precipitate of cerium perhydroxide was detected. Following stimulation with IL-5 for 15 min, the luminal part of intracytoplasmic vacuoles of both PMN and eosinophils, were coated with a layer of electron-dense material (Fig. 7c). Significant deposits of the reaction product were detected mainly in the contact zone between adjacent PMN or between PMN and eosinophils. There were no marked signs of degranulation in eosinophils upon stimulation with IL-5. In contrast, in the non-stimulated cells, little or no reaction product was present. No other cytoplasmic organelles were reactive for hydrogen peroxide.

Discussion

Human IL-5 directly stimulated a significant long-lasting chemiluminescence (CL) response in granulocyte preparations from patients with AD, but not in those from non-atopic controls. The effect was induced at concentrations of human IL-5 as low as 1 U/ml. Similar effects were observed when murine IL-5 was used, supporting the observation of cross-reactivity between these cytokines.[2-4] However, murine IL-5 was shown to have less biological activity, even at the highest concentrations tested. The time course showed a single peak, which is similar to the effects induced by the tumour promoter PMA. In contrast, GM-CSF and TNFα were found to induce double-peaked CL responses.[19–21] The CL response started after a short lag-phase which is probably due to transductional events following binding of IL-5 to its receptor. However, in comparison to GM-CSF or TNFα this lag-phase was significantly shorter.[11,12] At low concentrations of IL-5 there was a prolonged lag-phase with the peak appearing significantly later. This has not been found with CL responses induced by other cytokines. These results suggest a dose-dependent delay of IL-5-induced granulocyte activation that may be of physiological importance *in vivo*.

The relative specificity of the enhancement of the CL response by IL-5 for atopic diseases was confirmed by the similar response of patients with allergic rhinitis, whereas this was not found in granulocytes from patients with active psoriasis. Thus, the IL-5-induced effects in AD patients are not simply an epiphenomenon of inflammation or an additional sign of 'granulocyte hyperactivation' as suggested for psoriasis.[22]

In order to evaluate whether the capacity of IL-5 to stimulate granulocyte functions was limited to an enhancement of CL responses, its effect on the release of ECP as a measure of degranulation was investigated. However, no significant effect could be detected in any of the groups tested and the recently observed increase of ECP serum levels in AD patients[23] is probably not due to an IL-5-induced activation of eosinophils *in vivo*.

To determine whether eosinophils and/or PMN are the target of IL-5, its effect on granulocytes was investigated by ultrastructural techniques. Morphometric analysis demonstrated that, in contrast to normal controls, upon stimulation with IL-5 almost all the granulocytes from AD patients showed morphological signs of activation. Because the granulocyte preparations tested contained only 19 ± 5% eosinophils, these results clearly show that both PMN and eosinophils were activated. However, it was shown recently that highly purified PMN[7] did not respond to stimulation with IL-5. Moreover, no receptors for IL-5 were detected on highly purified PMN from normal controls.[24] It is possible that PMN are activated indirectly, probably by factors released by IL-5-activated eosinophils or by an IL-5 mediated, adherence-dependent mechanism.

Activated granulocytes showed a characteristic pattern in scanning EM very similar to that induced by GM-CSF in PMN.[20] The production of reactive oxygen species (ROS) in both PMN and eosinophils was confirmed by the detection of hydrogen peroxide production which was visualized by the detection of the electron-dense precipitate of cerium perhydroxide.[20] The reaction product was found in the intracytoplasmic vacuoles and particularly in the intercellular contact zone, suggesting a significant production of ROS by both cell types. Transmission EM revealed no significant signs of degranulation in the eosinophils. These results are in agreement with the functional assay systems. Because ROS production was only detected in PMN and eosinophils which were adjacent, it appears likely that an increase of adherence might be an essential prerequisite for the IL-5-induced CL response. Comparable effects were observed for the interaction of PMN with endothelial cells upon stimulation with TNFα.[25] Moreover, by this mechanism the production of ROS is probably localized at the site of inflammation.

The major effect of IL-5 on granulocytes of atopic patients may be due to a disease-specific preactivation of granulocytes in these patients. This hypothesis is further supported by the finding of an increased response to stimulation with other granulocyte-activating cytokines, in particular GM-CSF, in atopic patients. It is tempting to speculate that cytokines, produced locally by keratinocytes or epithelial cells,[26,27] could act on circulating eosinophils or PMN modulating their functional activity. PMN, prestimulated with GM-CSF or TNFα, respond to subsequent stimulation with the chemotactic peptide f-met-leu-phe with a significantly increased CL signal.[13,19,21] This effect is probably due to an increase in receptor number and affinity.[28] Because it has been shown recently that overnight incubation with GM-CSF significantly increases the IL-5 receptor affinity on eosinophils,[24] similar mechanisms could produce the observed effects. However, whether GM-CSF represents a 'priming stimulus' which is of relevance for atopic disease is as yet unknown. The potential role of cytokines in this process is further supported by reports identifying profound dysregulation within the cytokine network in AD.[29] Further studies are required to examine the mechanism of the IL-5-mediated effect on PMN.

In summary, this study demonstrates that IL-5 induces the activation of both eosinophils and PMN from atopic patients. In addition to its importance for the inflammatory response *in vivo* this phenomenon may possibly serve as a functional assay system for *in-vitro* allergy testing.

Acknowledgments

We thank A.Komann and C.Scholz for excellent technical assistance, and J.Krutmann, MD, for critical revision of the manuscript. This study was applied by a grant of the Deutsche Forschungsgemeinschaft.

References

1 Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation *in vitro*. *J Exp Med* 1985; **162:** 60–74.

2 Lopez AF, Begley CG, Williamson DJ *et al.* Murine eosinophil differentiation factor. *J Exp Med* 1986; **163:** 1085–99.

3 Harriman GR, Strober W. The immunobiology of interleukin-5. *Year Immunol* 1989; **5:** 160–77.

4 Sanderson CJ. Interleukin-5: An eosinophil growth and activation factor. *Dev Biol Stand* 1988; **69:** 23–9.

5 Azuma C, Tanabe T, Konishi M *et al.* Cloning of cDNA for human T-cell replacing factor (interleukin-5) and comparison with the murine homologue. *Nucl Acids Res* 1986; **14:** 9149–58.

6 Campbell HD, Tucker WQJ, Hort Y *et al.* Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin 5). *Proc Natl Acad Sci USA* 1987; **84:** 6629–33.

7 Lopez AF, Sanderson CJ, Gamble JR *et al.* Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988; **167:** 219–24.

8 Wang JM, Rambaldi A, Biondi A *et al.* Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989; **19:** 701–5.

9 Rothenberg ME, Petersen J, Stevens RL *et al.* IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. *J Immunol* 1989; **143:** 2311–16.

10 Plaut M. Antigen-specific lymphokine secretory patterns in atopic disease (commentary). *J Immunol* 1990; **144:** 4497–500.

11 Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 1989; **7:** 145–83.

12 Wierenga EA, Snoek M, de Groot C *et al.* Evidence for compartimentalization of functional subsets of CD4$^+$ T lymphocytes in atopic patients. *J Immunol* 1990; **144:** 4651–56.

13 Kapp A, Zeck-Kapp G. Activation of the oxidative metabolism in human polymorphonuclear neutrophilic granulocytes—the role of immuno-modulating cytokines. *J Invest Dermatol* 1990; **95:** 94S–99S.

14 Gleich GJ, Adolphson CR. The eosinophilic leukocyte: Structure and function. *Adv Immunol* 1986; **39:** 177–253.

15 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; Suppl **92:** 44–7.

16 Kapp A, Danner M, Luger TA *et al.* Granulocyte-activating mediators (GRAM): II. Generation by human epidermal cells. *Arch Dermatol Res* 1987; **279:** 470–7.

17 Kapp A, Luger TA, Maly FE, Schöpf E. Granulocyte-activating mediators (GRAM): I. Generation by LPS-stimulated mononuclear cells. *J Invest Dermatol* 1986; **86:** 523–8.

18 Edwards SW. Luminol- and lucigenin-dependent chemiluminescence of neutrophils: role of degranulation. *J Clin Lab Immunol* 1987; **22:** 35–9.

19 Kapp A, Zeck-Kapp G, Danner M, Luger TA. Human granulocyte–macrophage colony stimulating factor: An effective direct activator of human polymorphonuclear neutrophilic granulocytes. *J Invest Dermatol* 1988; **91:** 49–55.

20 Zeck-Kapp G, Kapp A, Riede UN. Activation of human polymorphonuclear neutrophilic granulocytes by immuno-modulating cytokines—an ultrastructural study. *Immunobiol* 1989; **179:** 44–55.

21 Kapp A, Zeck-Kapp G, Blohm D. Human tumor necrosis factor is a potent activator of the oxidative metabolism in human polymorphonuclear neutrophilic granulocytes (PMN)—Comparison with human lymphotoxin. *J Invest Dermatol* 1989; **92:** 348–54.

22 Christophers E. Leukocytes and skin disease: Conflicting patterns of inflammation in atopic dermatitis and psoriasis. *J Dermatol (Tokyo)* 1986; **13:** 408–16.

23 Kapp A, Czech W, Krutmann J, Schöpf E. Eosinophil cationic protein (ECP) in sera of patients with atopic dermatitis. *J Am Acad Dermatol* 1991; **24:** 555–8.

24 Chihara J, Plumas J, Gruart V *et al.* Characterization of a receptor for interleukin 5 on human eosinophils: variable expression and induction by granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1990; **172:** 1347–51.

25 Zeck-Kapp G, Kapp A, Busse R, Riede UN. Interaction of granulocytes and endothelial cells upon stimulation with tumor necrosis factor α—an ultrastructural study. *Immunobiol* 1990; **181:** 267–75.

26 Luger TA, Schwarz T. Epidermal cell derived cytokines. In: *Skin Immune System.* (JD Bos, ed.) Boca Raton, Florida: CRC press, 1989; 257–91.

27 Denburg JA, Otsuka H, Ohnisi M *et al.* Contribution of basophil/mast cell and eosinophil growth and differentiation to the allergic tissue inflammatory response. *Int Archs Allergy appl Immun* 1987; **82:** 321–6.

28 Weisbart RH, Golde DW, Gasson JG. Biosynthetic human GM-CSF modulates the number and affinity of neutrophil f-met-leu-phe receptors. *J Immunol* 1986; **137:** 3584–7.

29 Kapp A. Cytokines in atopic dermatitis. In: *Handbook of Atopic Eczema.* (Ruzicka T, Ring J, Przybilla B, eds) Berlin: Springer, 1991.

British Journal of Dermatology (1991) 125, 116–122. ADONIS 0007096391001805

In-vivo administration of recombinant IL-2 increases the number of Thy-1$^+$ dendritic epidermal cells

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Accepted for publication 25 January 1991

Summary

The number, morphology and response of Thy-1$^+$ dendritic epidermal cells to recombinant interleukin-2 (rIL-2) were investigated in young and aged mice. The Thy-1$^+$ dendritic cells in the aged mice continued to express T-cell receptor (TCR) γ/δ but differed morphologically from those of the young mice. The aged mice had 50% fewer cells than the young ones. In the rIL-2 treated mice all the Thy-1$^+$ cells were expressed as TCR γ/δ and exposure to rIL-2 increased the number of these cells in a time- and dose-dependent manner when given systemically and locally. In the aged mice daily injection of rIL-2 increased the number of Thy-1$^+$ dendritic cells within 2 weeks to almost that of young mice, however they had a lower response in the earlier stage. Nude mice showed no response to rIL-2.

Two types of dendritic cells are to be found in murine epidermis, Thy-1$^+$ cells that express a γ/δ T-cell receptor (TCR) on the surface[1–4] and Langerhans cells (LC) which are involved in antigen presentation.[5,6] These cells, together with keratinocytes, play an important role in the immunological regulation of the skin.[7] However, the equivalent of murine Thy-1$^+$ dendritic cells has not been found in any other species apart from mice. It is unknown as to whether immunoreactivity decreases with age, especially in relation to T-cell function, although in a recent report the number of LC were decreased in aged mice.[8] It was also shown that the administration of cytokines including interleukin 2 (IL-2) increased the number of Langerhans cells both *in vitro* and *in vivo*.

In this study, we have investigated whether there are morphological changes and differences in the number of Thy-1$^+$ dendritic cells and LC in young and aged mice. We also investigated whether the number of cells in young and aged mice could be increased by injecting recombinant IL-2 (rIL-2) *in vivo*, and whether this response differed with the age of the mice.

Methods

Animals

Mice aged 24–26 months, young 3-month-old and adult nude 12-month-old female mice were used in this study. They were C57BL/6 (H-2^b), BDF$_1$ (C57BL6/DBA2 F$_1$) and C3H/HeN(H-2^k) and were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. The skin samples were obtained from the ear or the foot pad. Epidermal sheets were obtained by incubating the skin with 3·8% ammonium thiocyanate and the sheets fixed in acetone at −20°C for 40 min and then washed in phosphate-buffered saline (PBS) as previously described.[9] The specimens were then stained with the monoclonal antibodies.

Monoclonal antibodies

The epidermal sheets were stained with the following monoclonal antibodies (MAbs): anti-mouse Thy-1, anti-mouse L3T4, anti-mouse Lyt2, anti-asialo GM1, anti-mouse Ia^k and anti-mouse Ia^d (all these antibodies were obtained from Becton Dickinson, Mountain View, CA, U.S.A.); 145–2C11 (anti-mouse CD3), UC7-13D5 (anti-mouse γ/δ TCR) (a gift from Dr J.A.Bluestone, University of Chicago, U.S.A.); H57–597 (anti-mouse α/β TCR) (Pharmingen, San Diego, CA, U.S.A.); 3C7 (anti-IL-2α receptor) (a gift from Dr H.Yagita, Juntendo University, Japan); and NLDC-145 (anti-mouse LC) (a gift from Dr S.I.Katz, National Institute of Health, Bethesda, U.S.A.). FITC- or TRITC-conjugated anti-mouse or hamster IgG antibodies were used for the secondary staining.

Double-staining method

Epidermal sheets from the ear were examined for both Ia and Thy-1 antigens using a double-staining method.

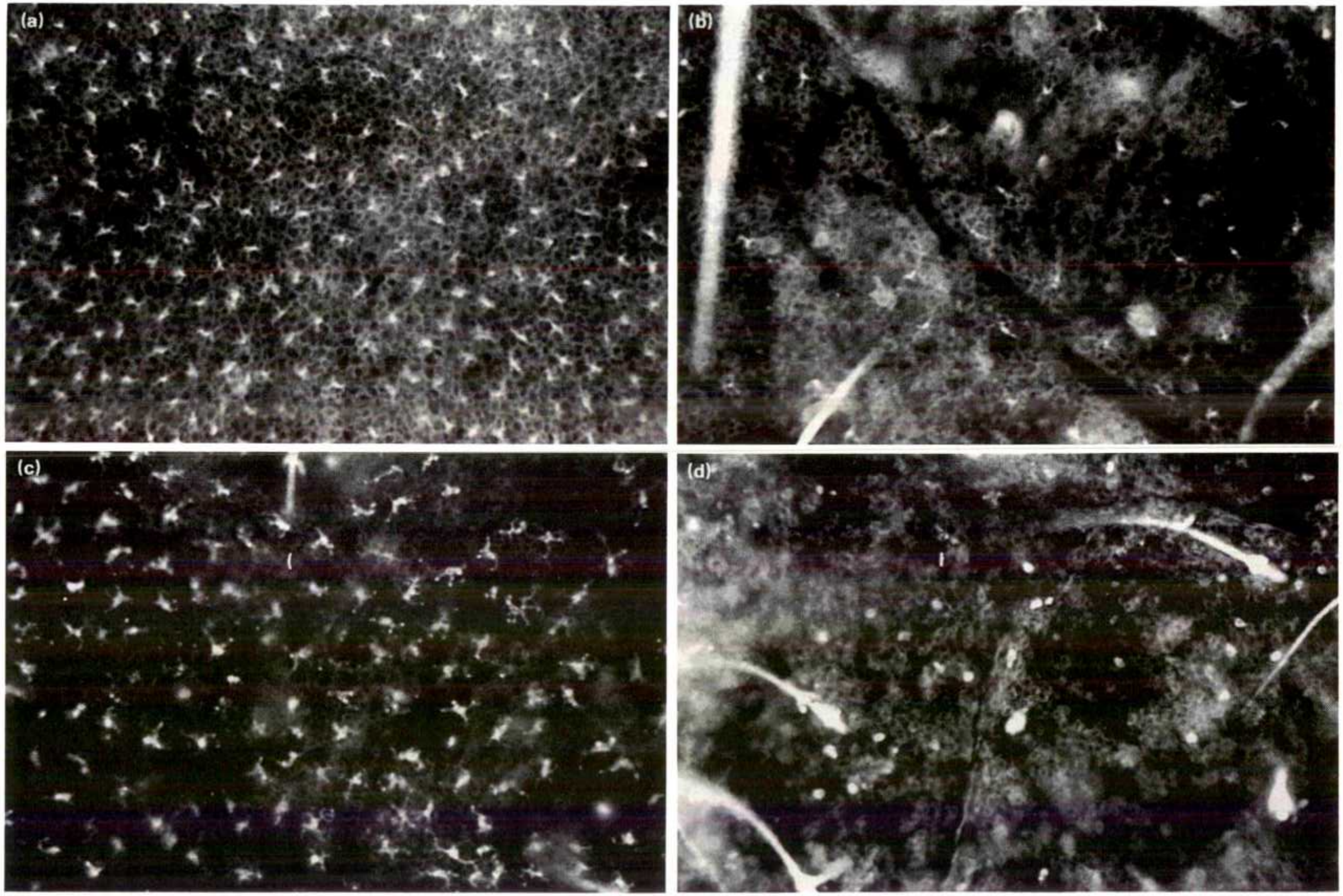

Figure 1. Epidermal sheets of young and aged mice. (a) Langerhans cells in young and (b) aged mice stained with anti-Ia. (c) Thy-1 $^+$ dendritic cells in young and (d) aged mice stained with anti-Thy-1 (× 160).

The epidermal sheets were fixed in acetone and were then incubated with an anti-mouse Ia antibody for 6 h, washed in PBS and then incubated with a TRITC-labelled anti-mouse IgG. These sheets were then incubated with mouse serum to block the antibody and then washed in PBS and incubated with FITC-labelled anti-mouse Thy-1 antibody for 6 h.

In-vivo administration of rIL-2

Recombinant IL-2 (a gift from Takeda Pharmaceutical Company, Japan) in different concentrations of 0·5, 1 and 5 μg in minimum essential medium (MEM) were injected into the abdominal cavity or into the foot pad of the animals daily for 2 weeks. Epidermal sheets were obtained at the end of the first and second weeks.

Enumeration of the stained cells

The number of stained cells in the epidermal sheet was determined using fluorescence microscopy. Three 1-mm^2 areas of each epidermal sheet were randomly chosen. Using a calibrated eyepiece the number of stained cells per 1 mm^2 was determined. For statistical analysis, the Student *t*-test was used and a *P* value of less than 0·05 was considered to be significant.

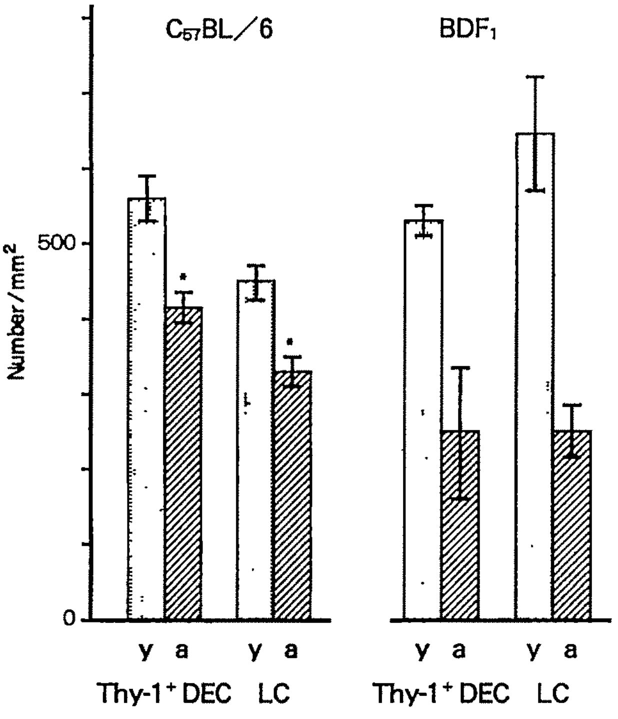

Figure 2. Number of Thy-1$^+$ dendritic cells and Langerhans in the ears of young (y) and aged (a) mice. Results are mean ± of three experiments (* $P<0{\cdot}05$).

Results

Cell morphology and numbers in young and aged mice

The number of Thy-1$^+$ dendritic cells and Langerhans cells in the aged mice were less than those in the young mice, and were also morphologically different with shorter dendrites that had a more circular shape (Fig. 1). The number of Thy-1$^+$ dendritic cells and LC was reduced in both types of aged mice, especially in the BDF$_1$ that had about 50% fewer cells than the young mice (Fig. 2). However, there were no changes in the expression of CD3 antigen and TCR γ/δ on the Thy-1$^+$

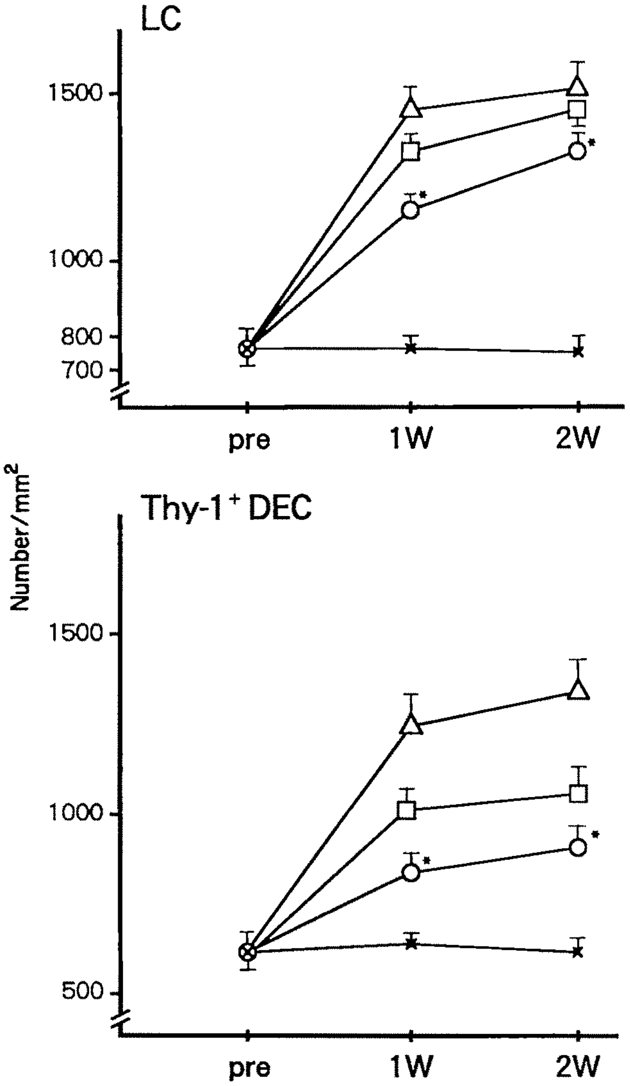

Figure 3. (a) and (b) Number of Langerhans cells and Thy-1$^+$ dendritic cells before and 1 and 2 weeks after daily injections of recombinant IL-2 into the abdominal cavity of young mice. Results are mean ± SE from three experiments (*$P<0{\cdot}05$). ×, MEM; ○, 0·5 μg/day IL-2; □, 1 μg/day IL-2; △, 5 μg/day IL-2.

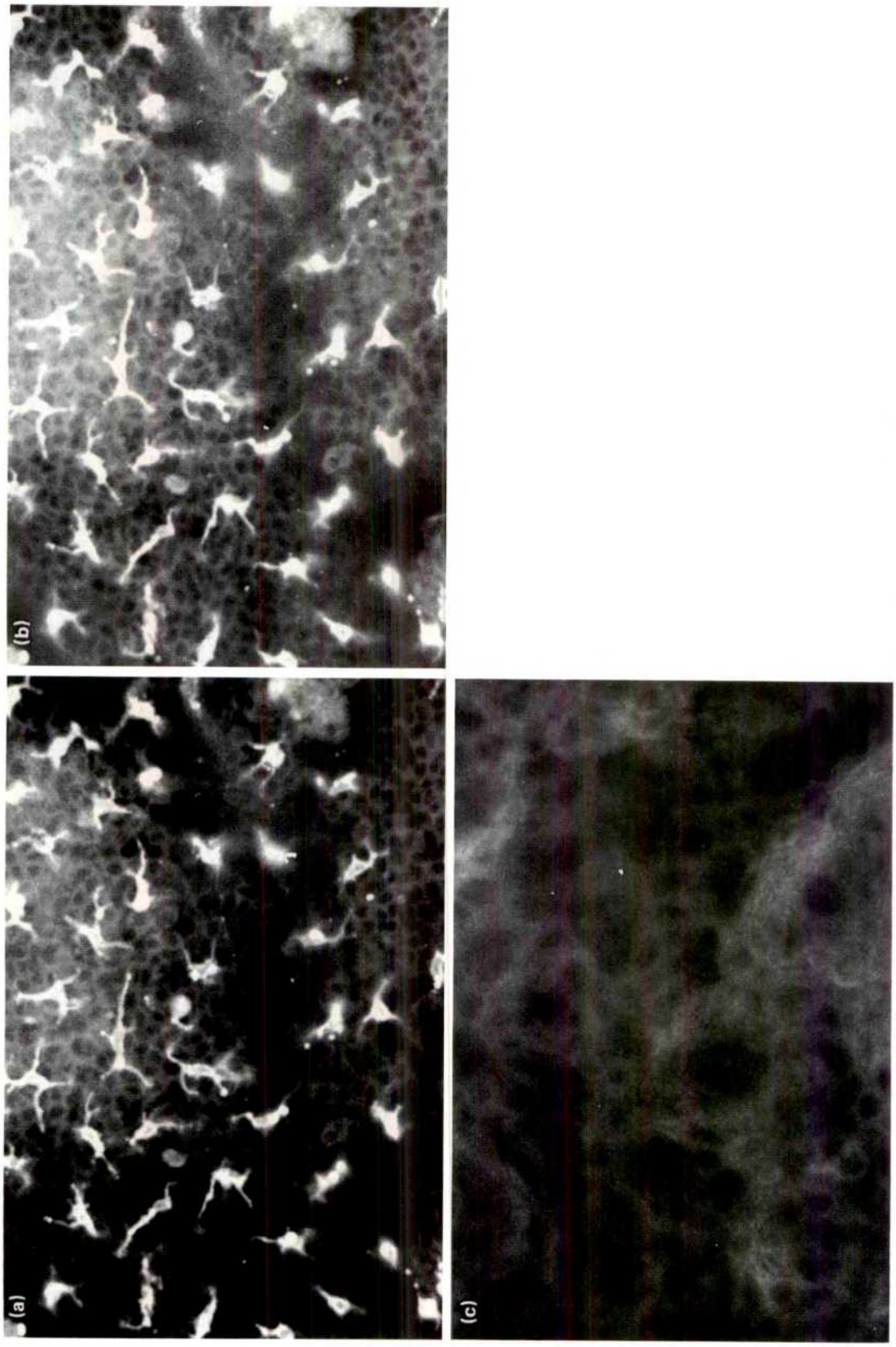

Figure 4. Epidermal sheets from young mice stained with (a) anti-Thy-1, (b) anti-γ/δ TCR and (c) anti-α/β TCR antibodies using the double-staining method (× 320).

dendritic cells that continued to be expressed at all the ages.

Effect of injecting rIL-2 into the abdominal cavity of young mice

The injection of rIL-2 caused the number of epidermal cells stained with anti-Thy-1 and anti-Ia to increase in a time- and dose-dependent manner (Fig. 3). The epidermis of the control and the rIL-2 injected groups were also stained with NLDC-145 antibodies, being another surface marker of LC, and the number of NLDC-145$^+$ cells was similar to that of Ia$^+$ cells. No double-stained cells were found using the anti-Thy-1 and anti-Ia antibodies on the epidermal sheets obtained from the ears of injected mice at the end of the second week. When the epidermis was stained with anti-α/β TCR and anti-γ/δ TCR antibodies, we found that almost all the Thy-1$^+$ dendritic cells expressed TCR γ/δ and none expressed TCR α/β (Fig. 4). The epidermal sheets were also stained with anti-L3T4, anti-Lyt2 and anti-IL-2α-receptor antibodies, but did not show any positive cells. The injection of rIL-2 appeared to increase the number of Thy-1$^+$ dendritic cells that expressed both CD3 and TCR γ/δ.

Effect of injecting rIL-2 into the foot pads of young mice

Different concentrations of rIL-2 (0·1, 0·5, 1 and 5 μg) were injected daily into the left foot pads of young mice for up to 2 weeks. Epidermal sheets were obtained from

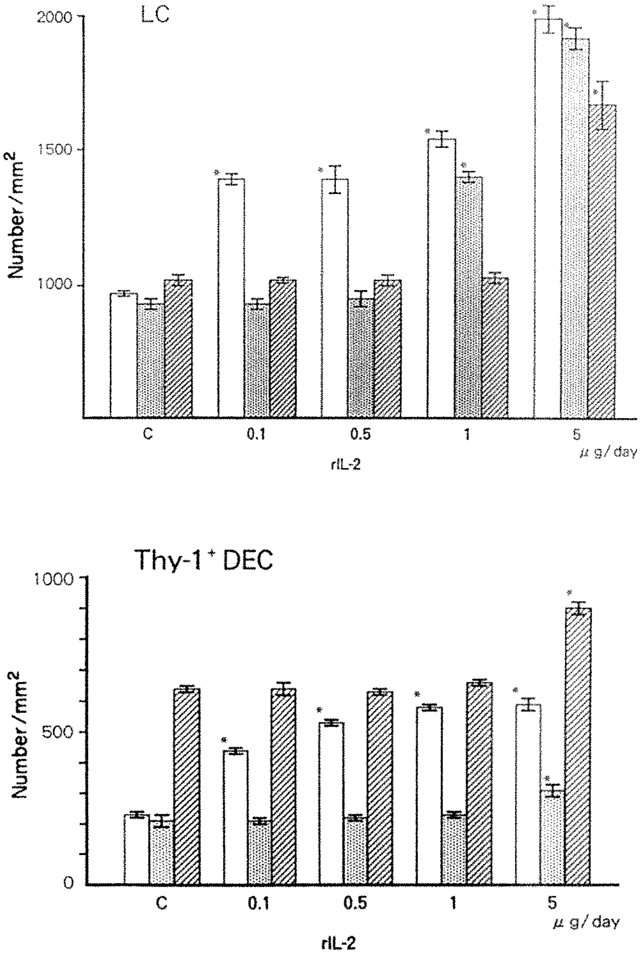

Figure 5. Number of Langerhans cells and Thy-1$^+$ dendritic cells 2 weeks after daily injection of different doses of recombinant IL-2 into the foot pad of young mice. Results are mean ± SE from three experiments (*$P<0{\cdot}05$). □, left foot; ▩, right foot; ▨, ear.

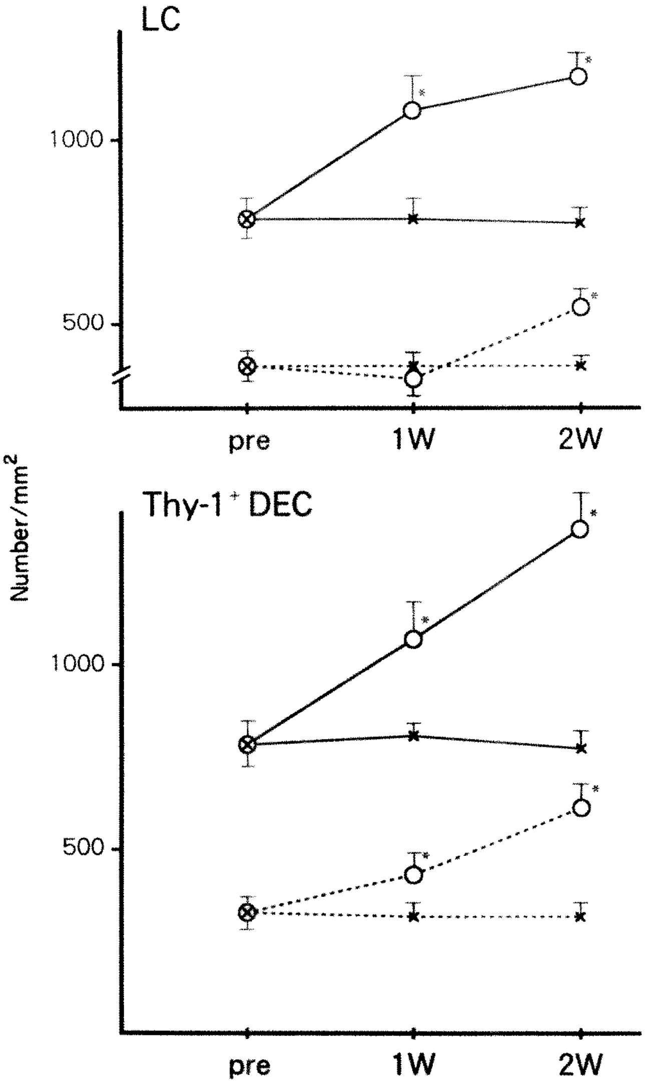

Figure 6. Response to daily injections of recombinant IL-2 for 2 weeks in young and aged BDF$_1$ mice. ○——○, young mice given 5 μg rIL-2; ×——×, young mice given MEM; ○- - -○, aged mice given 5 μg rIL-2; ×- - -×, aged mice given MEM. Results are mean ± SE from three experiments (*$P<0{\cdot}05$).

the left and right foot pads and the ears of these animals. With the higher concentrations of rIL-2 that were injected, the number of anti-Thy-1 and anti-Ia stained cells in all three areas increased in a time- and dose-dependent manner, although the greatest increase was seen in those sheets obtained from the injected left foot pad. However, when rIL-2 was injected at lower doses the number of stained cells increased only locally (Fig. 5).

Comparison of response to rIL-2 between young and aged and nude mice

The highest dose of rIL-2 (5 μg/day) was injected into the abdominal cavity of young, aged and nude mice. The rate of increase of LC and Thy-1$^+$ dendritic cells in aged mice was similar to that of the young mice at the second week, but was less in the first week (Fig. 6).

Discussion

Thy-1$^+$ dendritic cells are to be found only in murine epidermis and they express CD45, CD3 and asialo GM1, but lack CD5, CD8, CD4 and Ia antigens.[10,11] The T-cell receptor found on the Thy-1$^+$ dendritic cell is the γ/δ heterodimer rather than the α/β heterodimer that is expressed on ordinary T cells. Recent studies[12,13] have shown that the precursors of Thy-1$^+$ dendritic cells are a population of foetal thymocytes on day 15, because these cells expressed the TCR of $V_{\gamma 5}$-V_{δ} gene segments that are identical to Thy-1$^+$ dendritic cells.[13,14]

Although the role of Thy-1$^+$ dendritic cells remains unknown, it has been postulated[15,16] that γ/δ T cells, including Thy-1$^+$ dendritic cells, show cytotoxic potential and function as a first line of immunological defence. Thy-1$^+$ dendritic cells express perforin[17] that is a pore-forming protein thought to be involved in cell-mediated cytolysis. In addition these cell lines or clones exhibit MHC-unrestricted cytotoxic activity.[18,19]

A study of Langerhans cells, another type of dendritic immunocompetent cells, in murine epidermis showed a decrease in number with age and this recovered when certain cytokines were administered *in vivo*.[8] The number of Thy-1$^+$ dendritic cells was not mentioned in this study.

Our study has shown that the number of Thy-1$^+$ dendritic cells as well as LC in the aged mice were lower than in the young animals, and the number of these cells was increased following injections of IL-2. Their cell-surface markers remained unchanged.

Recently, Ishida *et al.*[20] developed transgenic mice that carried the cDNA of human IL-2 and also expressed human IL-2 mRNA in the skin. These workers found that there was a marked increase in Thy-1$^+$ dendritic cells in the epidermis in these animals. These results together with our findings suggest that IL-2 can increase the number not only of LC but also of Thy-1$^+$ dendritic cells in the epidermis. However, IL-2 receptor is not generally found on the surface of Thy-1$^+$ dendritic cells, and this was not observed in our study using immunofluorescence even after the injection of IL-2 into the skin. These findings suggest either that Thy-1$^+$ dendritic cells respond to IL-2 through the IL-2β receptor, whose expression is induced by IL-2 or other cytokines, or that these cells respond to IL-2 indirectly through the stimulation of other cells to produce cytokines. We are unable at present to find a monoclonal antibody to the IL-2β receptor in mice.

There are also several possible mechanisms by which the number of LC and Thy-1$^+$ dendritic cells are increased following the injection of IL-2. The Thy-1$^+$ dendritic cells may proliferate in the epidermis, and there may be a significant population of precursor cells and IL-2 may induce the expression in these of Thy-1 or γ/δ TCR. Also Thy-1$^+$ dendritic cells may be present in other tissues and migrate into the epidermis following the injection of IL-2. This last seems to be unlikely, because Thy-1$^+$ dendritic cells have only been found in the epidermis of adult mice.[21,22] Also in this study the number of Thy-1$^+$ cells was increased by the local injection of small doses of IL-2 which did not affect the distant skin.

Acknowledgments

We thank Dr K.Hirokawa for providing the aged mice. This work was supported by a grant from the Ministry of Education, Science and Culture, Japan.

References

1 Shimada S, Caughman SW, Bluestone JA *et al.* Freshly isolated Thy-1$^+$ dendritic epidermal cells express T cell receptor $\gamma\delta$-CD3. *J Dermatol Sci* 1990; **1:** 459–64.

2 Koning F, Stingl G, Yokoyama WM *et al.* Identification of a T3 associated $\gamma\delta$ T cell receptor on Thy-1$^+$ dendritic epidermal cell lines. *Science* 1987; **236:** 834–7.

3 Stingl G, Koning F, Yamada H *et al.* Thy-1$^+$ dendritic epidermal cells express T3 antigen and the T-cell receptor γ chain. *Proc Natl Acad Sci USA* 1987; **84:** 4586–90.

4 Kuziel WA, Takashima A, Bonyhadi M *et al.* Regulation of T cell receptor γ-chain RNA expression in murine Thy-1$^+$ dendritic epidermal cells. *Nature* 1987; **328:** 263–6.

5 Stingl G, Tamaki K, Katz SI. Origin and function of epidermal Langerhans cells. *Immunol Rev* 1980; **53:** 149–74.

6 Shimada S, Caughman SW, Sharrow S *et al.* Enhanced antigen-presenting capacity of cultured Langerhans' cell is associated with markedly increased expression of Ia antigen. *J Immunol* 1987, **139:** 2551–5.

7 Shimada S, Katz SI. The skin as an immunologic organ. *Arch Pathol Lab Med* 1988; **112:** 231–4.

8 Belsito DV, Epstein SP, Schulz JM *et al.* Enhancement by various cytokines or 2-β-mercaptoethanol of Ia antigen expression on Langerhans cells in skin from normal aged and young mice. Effect of cyclosporine A. *J. Immunol* 1989; **143:** 1530–6.

9 Breathnach SM, Katz SI. Thy-1$^+$ dendritic cells in murine epidermis are bone marrow-derived. *J Invest Dermatol* 1984; **83:** 74–7.

10 Tschachler E, Schuler G, Hutterer J *et al.* Expression of Thy-1 antigen by murine epidermal cells, *J Invest Dermatol* 1983; **81:** 282–5.

11 Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW Thy-1$^+$ antigen bearing dendritic cells populate murine epidermis. *J Invest Dermatol* 1983; **81:** 286–8.

12 Havran WL, Allison JP. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature* 1988, **335:** 443–5.

13 Lafaille JJ, Decloux A, Bonneville M *et al.* Junctional sequences of T cell receptor $\gamma\delta$ genes: Implications for $\gamma\delta$ T cell lineages and for a novel intermediate of V-(D)-J joining. *Cell* 1989; **59:** 859–70.

14 Ito K, Bonneville M, Takagaki Y *et al.* Different $\gamma\delta$ T cell receptors are expressed on thymocytes at different stages of development. *Proc Natl Acad Sci USA* 1989; **86:** 631–5.

15 Modlin RL, Pirmez C, Hofman FM *et al.* Lymphocytes bearing antigen specific $\gamma\delta$ T cell receptors accumulate in human infectious disease lesion. *Nature* 1989; **339:** 544–8.

16 Janeway CA. Frontiers of the immune system *Nature* 1988; **333:** 804–6.

17 Kobata T, Shinkai Y, Iigo T *et al.* Thy-1 positive dendritic epidermal cells contain a killer protein perforin. *Int Immunol* 1990, **2:** 1113–6.

18 Nixon-Fulton JL, Hackett J Jr, Bergstresser PR *et al.* Phenotypic heterogeneity and cytotoxic activity of Con A and IL-2-stimulated Thy-1$^+$ epidermal cells. *J Invest Dermatol* 1987; **91:** 62–8

19 Shimada S, Caughman SW, Minami Y *et al.* Molecular and biochemical characterization of the T cell receptor on Thy-1$^+$DEC in athymic nude mice. *Clin Res* 1987; **35:** 717A

20 Ishida Y, Nishi M, Honjo T *et al.* Effects of the deregulated expression of human interleukin-2 in transgenic mice *Int Immunol* 1989; **1:** 113–20.

21 Itohara S, Nakanishi N, Kanagawa O *et al.* Monoclonal antibodies specific to native murine T cell receptor $\gamma\delta$: Analysis of $\gamma\delta$ T cells during thymic ontogeny and in peripheral lymphoid organs. *Proc Natl Acad Sci USA* 1989; **86:** 5094–8.

22 Itohara S, Farr AG, Lafaille JJ *et al* Homing of a $\gamma\delta$ thymocyte subset with homogeneous T cell receptors to mucosal epithelia. *Nature* 1990; **343:** 754–7.

British Journal of Dermatology (1991) **125**, 123–129.

ADONIS 000709639100181I

Measurement of human hair growth by optical microscopy and image analysis

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Accepted for publication 27 February 1991

Summary We have developed a quantitative method for measuring hair growth using optical microscopy and image analysis, and have used this to investigate the rate of growth in subjects with and without alopecia. The hairs were cut from an area 7–8 mm in diameter and 24 h and 72 h later, images of the areas were obtained using an optical microscope and were recorded on a video disc. Measurements of the regrowing hairs, placed parallel to the scalp using a glass slide attached to the front of the microscope, were made using the image analyser.

In subjects with little or no baldness there was a clear difference between fast-growing hairs and resting or slow-growing hairs. However, in subjects with alopecia there was no such difference and the growth rate of all the hairs showed a continuous distribution. Using this method other parameters such as the number of hairs per unit area and hair diameter as well as grouping of the hairs could be measured.

Quantitative methods for the measurement of human hair growth are necessary to assess how effective treatments are in promoting hair growth, and these must be accurate and simple to carry out. Several methods have been used to assess the rate of hair growth, and these include the capillary method,[1] radioisotope labelling[2] and fluorescent dyes.[3] Alternative methods for measuring the ratio of anagen and telogen hairs include plucking hair samples,[4–6] tissue trichograms,[7] phototrichograms,[8–11] and image analysis.[12,13]

The phototrichogram method provides much information as to the stage of the hair growth cycle, but analysis of the photographs is time consuming. Improved methods measure the binary image with an image analyser but the results are affected by the binary level and lighting conditions, and it is difficult to evaluate hairs that overlap.

We have developed a new method that involves using an optical microscope and image analyser, but without binary processing. Our method allows the measurement of the rate of hair growth, the ratio of resting and slow-growing hairs, the diameter of the hairs, and the number of hairs per unit area with an analysis of the hair groupings at the same time.

Methods

Cutting of hair

Hairs were cut using scissors in a circular area with a diameter of 7–8 mm (Fig. 1).

Video recording and image analysing system

An outline of the system for the video recording and image analysis used in this study is shown in Figure 2. The optical microscope used (Video microscope KC-510, Wilson Co., Japan) gave a × 30 magnification and a still

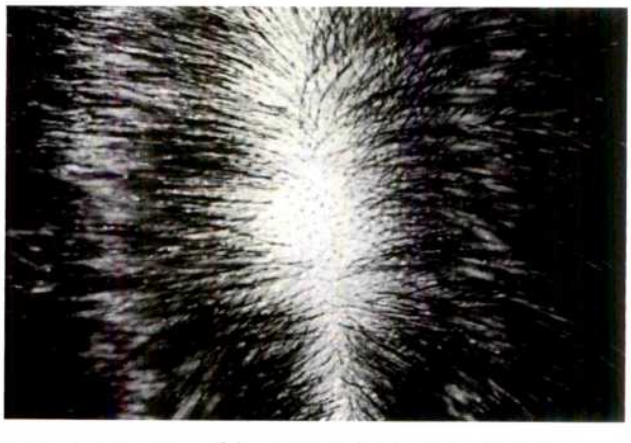

Figure 1. Appearance of the site immediately after cutting hair.

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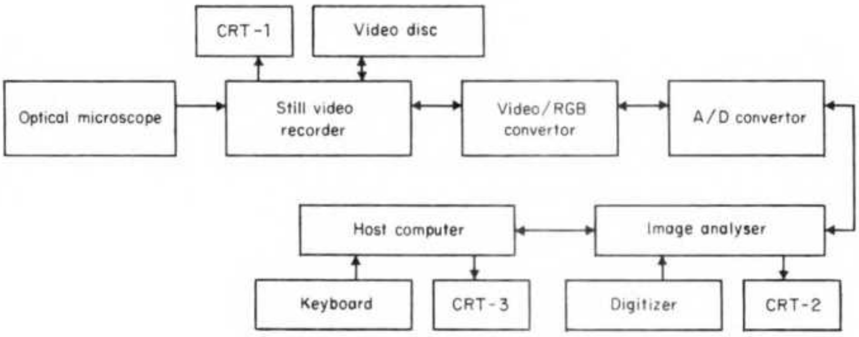

Figure 2. Outline of the image analysis system.

video recorder (RR-551, Canon Co., Japan) was used to record the areas of cut hair on to disc. The recorded images were then processed with a video/RGB signal conversion and an analogue/digital conversion and incorporated into the image analyser (NEXUS6400, Nexus Co., Japan). Measurements were made from the converted images with commands from the host computer and digitizer.

The hairs were placed parallel to the scalp using a glass slide that was attached at the front of the optical microscope during the video recordings of the sites from where the hair had been cut. The time taken for image input from each site including the site adjustment was less than 3 min.

Measurement intervals

The longer the interval between the measurements of hair growth, the more precise these were in determining the rate of growth of hair. Measurements were made 1 and 3 days after the hair was cut as shown in Figure 3.

Identification of each hair

It was necessary to identify each hair in the serial images of the same site. The appearances of the hairs 1 and 3 days after cutting are shown in Figure 4. A code name was allocated for the same hair in the images taken on both day 1 and day 3 following cutting, and the serial images with codes were recorded on the video disc.

Using this method, hairs at the same site could be measured and recorded repeatedly with an interval of 6 months as shown in Figure 5.

Measurements of hair length and diameter

The hair length was measured 1 and 3 days after cutting and the diameter 1 day afterwards (Fig. 6). The length of each hair was measured on the monitor (CRT-2) by designating two points with the digitizer, one being where the hair emerged and the other at its tip. The

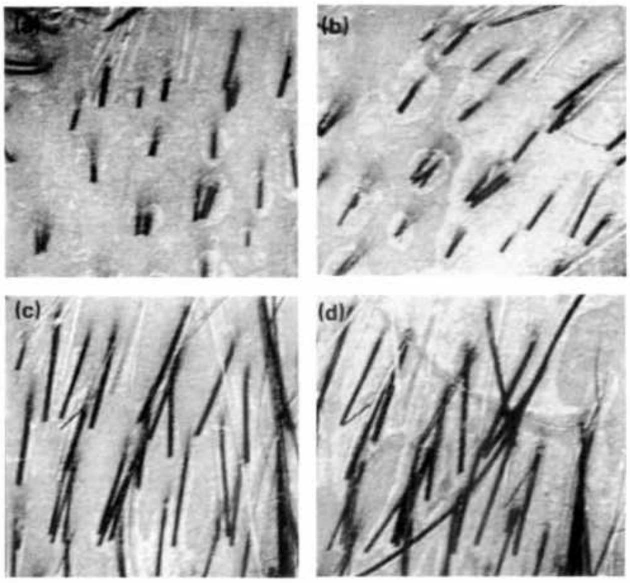

Figure 3. Changes in hair length in the same region (a) immediately following cutting and (b) 1 day, (c) 3 days and (d) 4 days later.

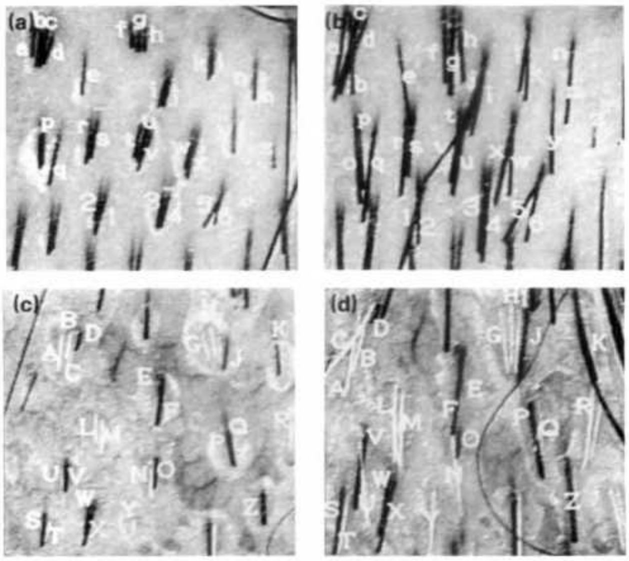

Figure 4. Assignment of codes to the same individual hairs in images taken 1 day (a, c) and 3 days (b, d) after cutting. (a) and (b) include black hair only and (c) and (d) include black and grey hairs.

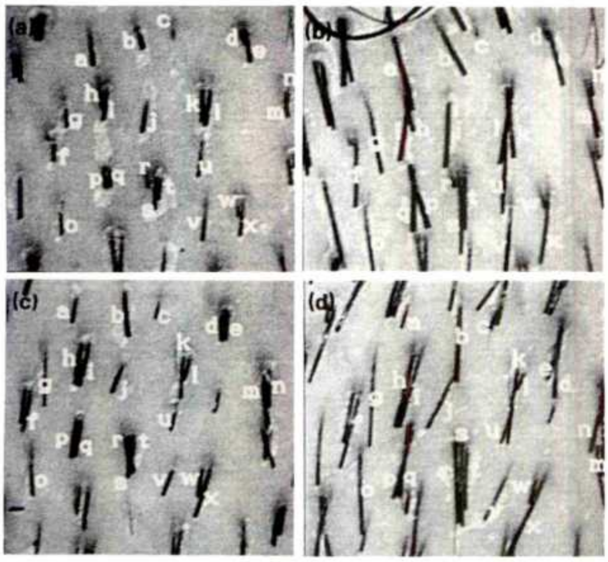

Figure 5. Hair growth images of the same site recorded repeatedly over 6 months. (a) 1 day after cutting and (b) 6 months later. (c) 3 days after cutting and (d) 6 months later.

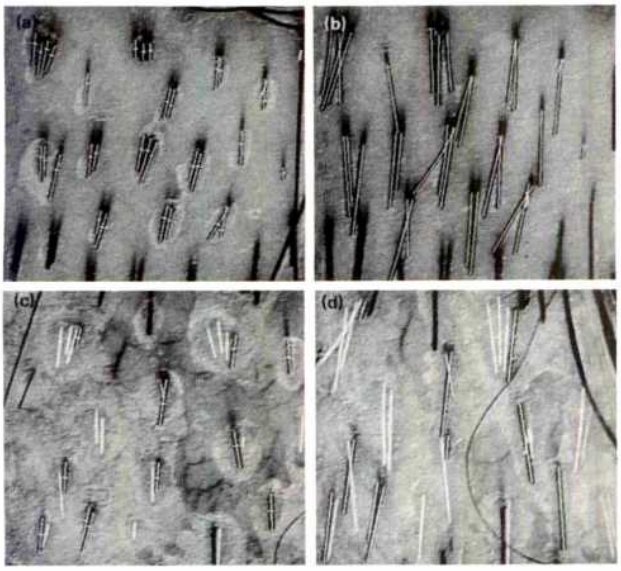

Figure 6. Measurement of hair length and diameter of black hairs only (a, b) and of black and grey hairs (c, d). Images taken 1 day (a, c) and 3 days after cutting (b, d). Each white line shows both hair length and diameter.

diameter of each hair was also measured on the monitor designating both sides of each hair with the digitizer. The reproducibility of the measurements of hair length and diameter were tested and 10 images were recorded at 1 and 3 days after cutting the hair in the temporal region of a male subject without alopecia. The standard deviation and coefficient of variation are shown in Table 1.

Table 1. Standard deviations and coefficients of variation in the measurements of hair length (24 and 72 h after hair cut) and diameter (24 h after). Measurements of 20 hairs were carried out 10 times

Average values in 20 hairs	Hair length		Hair diameter
	24 h	72 h	
Mean (μm)	722	1690	73
Standard deviation (μm)	25	34	6
Coefficient of variation (%)	3·4	2·0	8·4

Hair counts per unit area

The number of hairs in a unit area of 1 cm^2 was calculated from the images taken 1 day after cutting, and these hairs were divided into those with a diameter <40 μm and those with a diameter $\geqslant 40$ μm.

Analysis of hair groupings

Adjacent hairs were collected into a group according to the arrangement of the follicular openings, and the ratios of one-hair, two-hair, three-hair and four-hair groups to the total number of hairs were calculated.

Results

Comparison of method with previous reports

The data obtained with our present method were compared with those previously reported and were similar, as shown in Table 2.

Measurement of hair growth

The patterns of rate of hair growth in a healthy male without baldness is shown in Figure 7 and in three other subjects in Figure 8. Most of the hairs showed a growth rate of $\geqslant 0{\cdot}2$ mm per day, and those hairs having a slower growth rate were mainly in the vertex region rather than in the temporal area.

Two subjects with some baldness showed a discontinuity in the rate of hair growth (Fig. 9). In both the subjects with no and slight baldness, hairs that had a slow growth rate could be readily distinguished from those with a fast rate of growth.

In patients with alopecia the pattern of the rate of hair growth showed considerable variation and the rate a more continuous distribution (Figs 10 and 11).

It was apparent that in the non-bald subjects and those with only a slight degree of baldness, the rate of

Table 2. Hair count, hair growth rate, diameter and ratio of thin hair ($< 40\ \mu m$) in non-bald subjects: comparison with previous reports

Author	Technique	Subjects (region)	Number/cm²	Growth rate µm/day	Diameter µm	Ratio of thin hair
Barman[5]	TG+OD	17	223	344	—	—
Saitoh[8]	PTG	3	—	440	—	—
Fiquet[11]	PTG	5 (Vertex)	200–325	—	—	—
Rushton[6]	TG+OD	10 (Frontal)	312±15	—	68±2	8·5±1
Friedel[10]	PTG	5 (Vertex)	204±9·9	350±30	76±5	9·2±1·8
Present method	PTG	10 (Vertex) 27–48 years	181±21	313±60	67±6	12·4±4·9

OD, direct observation; TG, trichogram; PTG, phototrichogram.

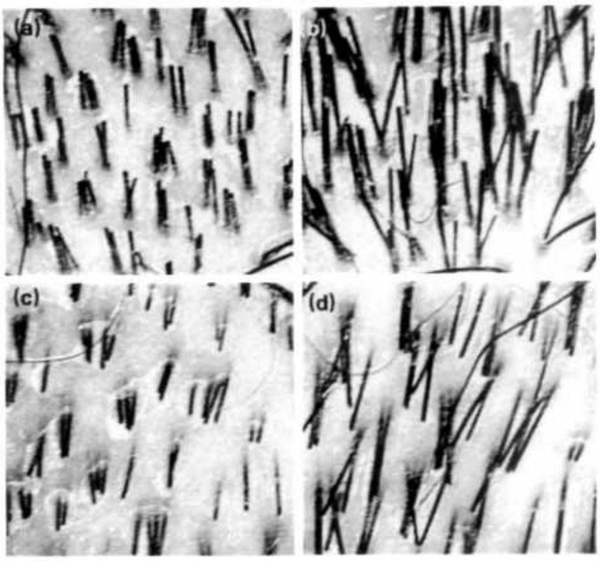

Figure 7. Typical images from the vertical (a, b) and temporal region (c, d) of a non-bald subject. Images taken 1 day (a, c) and 3 days after cutting (b, d).

growth of hairs could be divided into two groups, one that had a slow growth rate and the other that was fast. From our results and hair growth studies in the macaque monkey,[7] we classified hairs that had a growth rate of $\geqslant 0{\cdot}2$ mm/day as fast growing and those $< 0{\cdot}2$ mm/day as resting or slow-growing hairs.

Studies of the hair growth in subjects with alopecia

In contrast to the non-bald subjects, those with alopecia had, in addition to the slower rate of growth of hair, a higher ratio of resting and slow-growing hairs and a smaller average hair diameter and lower counts of thick hairs and more thin hairs. There was also a higher ratio of hairs growing singly (Table 3).

Discussion

Our studies have shown that using this method, in those with little or no baldness there was a clear separation

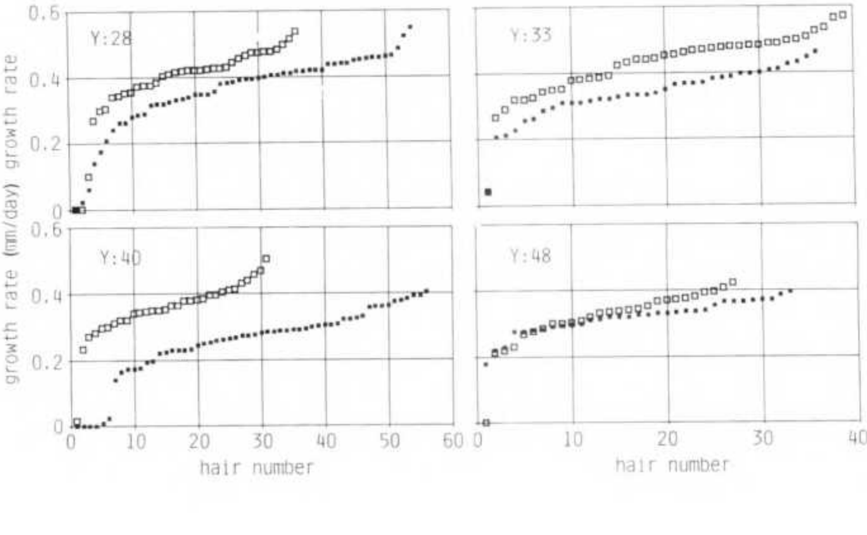

Figure 8. Patterns of hair growth rate in four non-bald subjects. Each point represents the hair growth rate of an individual hair, ordered into a sequence from lowest growth rate (left) to fastest growth (right). □, temporal region; ■, vertical region.

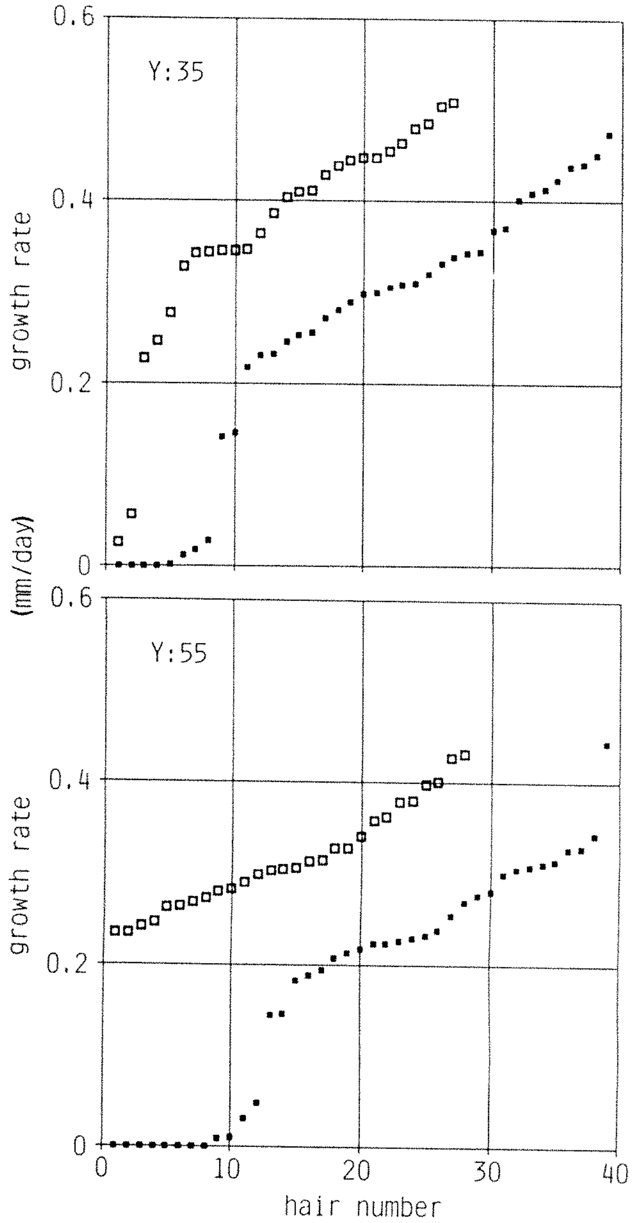

Figure 9. Patterns of hair growth in two subjects with slight baldness. Each point represents the hair growth rate of an individual hair. □, temporal region; ■, vertical region.

between those hairs that were fast growing and those that were resting/slow growing. In many of the subjects with alopecia this difference was not apparent and there was no sudden rise in the growth rate, but a more continuous distribution as regards growth.

In those subjects with no baldness almost all the hairs were in the anagen stage of the hair cycle. However, in those with baldness it was difficult to assess what stage in

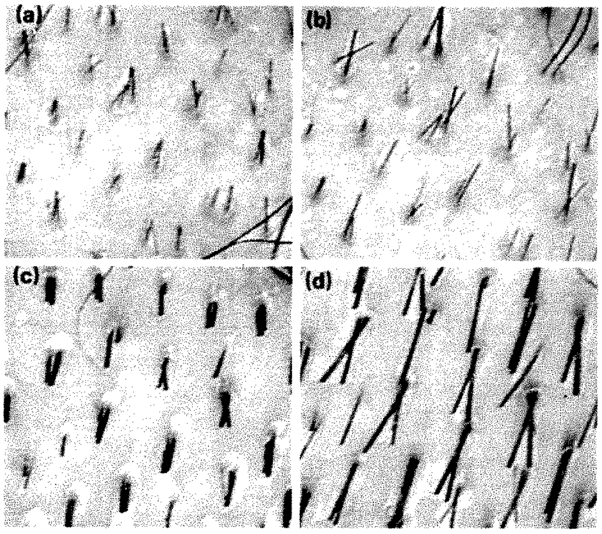

Figure 10. Typical images from the vertical (a, b) and temporal region (c, d) of a subject with alopecia. Images taken 1 day (a, c) and 3 days after cutting (b, d).

Table 3. Measurement of six parameters of hair growth in the vertical region of 10 non-bald subjects and 10 subjects with alopecia

Subject	Age	V	NA	DI	TH	VH	1/G
No baldness							
H1	27	309	23·8	73	155	30	6·9
H2	42	301	17·2	58	164	34	16·1
H3	40	250	23·2	57	147	32	6·5
H4	42	466	8·5	69	202	17	20·6
H5	37	264	7·5	71	174	6	1·1
H6	27	289	11·1	62	177	21	4·3
H7	37	313	11·4	71	123	30	29·0
H8	48	320	3·0	73	145	17	6·3
H9	46	277	9·4	71	138	19	20·0
H10	47	345	2·9	63	160	15	7·4
Mean	39·3	313·4	11·8	66·8	158·5	22·1	11·8
SD	7·5	60·4	7·4	6·2	22·4	8·6	9·0
Alopecia							
A1	43	151	59·3	35	64	104	37·9
A2	44	110	78·4	31	26	94	12·5
A3	41	75	83·8	37	57	128	32·2
A4	35	147	53·1	48	98	49	27·9
A5	46	93	81·3	25	13	140	25·8
A6	45	106	75·9	32	19	66	53·9
A7	48	147	65·5	46	119	55	26·7
A8	39	83	73·3	42	96	68	16·6
A9	44	78	79·1	28	17	123	49·3
A10	45	101	79·2	37	89	140	24·8
Mean	43·0	109·1	72·9	36·1	59·8	96·7	30·8
SD	3·8	29·4	10·2	7·5	39·4	35·4	13·1

V, hair growth rate (μm/day); NA, ratio of resting and slow growing hair (%); DI, hair diameter (μm); TH, counts of thick hairs (≥ 40 μm) per 1 cm^2; VH, counts of thin hairs (< 40 μm) per 1 cm^2; 1/G, ratio of hairs grown singly (%).

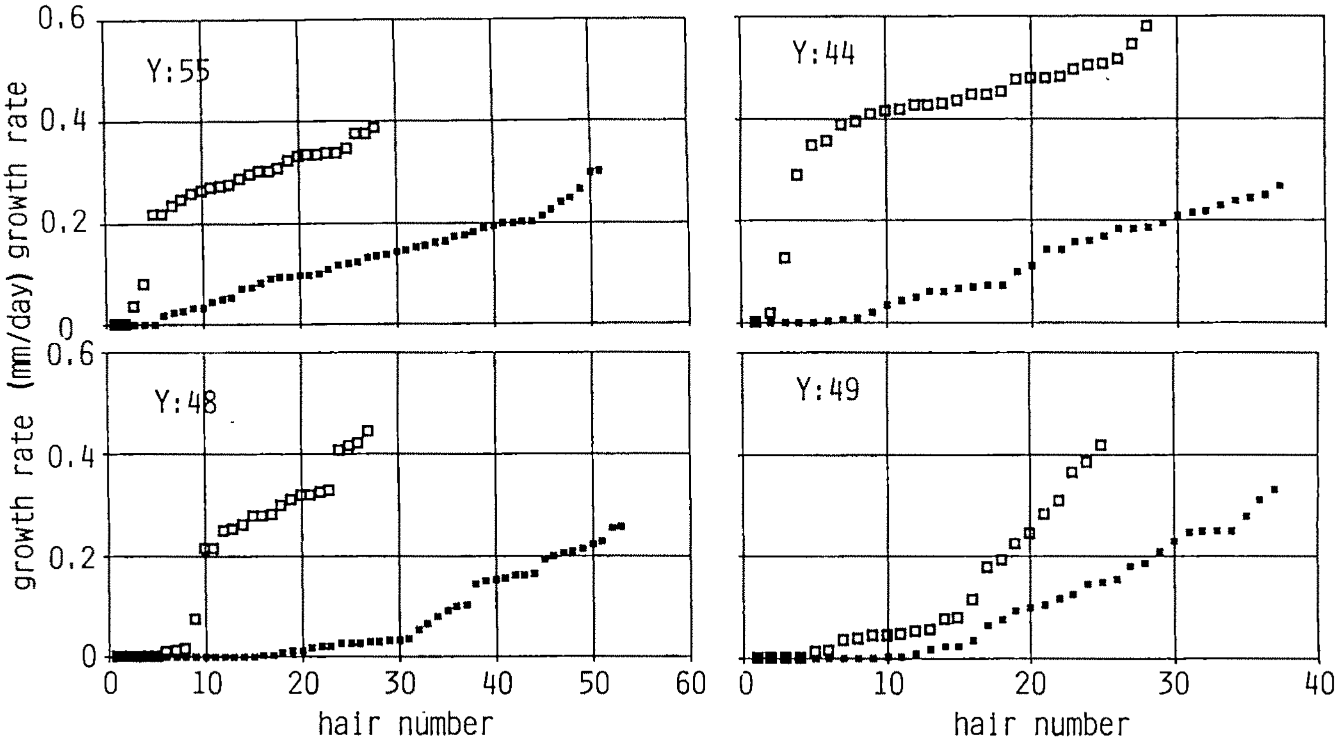

Figure 11. Patterns of hair growth in four subjects with alopecia. Each point represents the hair growth rate of an individual hair. □, temporal region; ■, vertical region.

the cycle each hair was in, because of a continuous distribution in rate of hair growth.

The factors that affect hair loss are considered to include a shortening of the hair cycle and a vellus transformation of the terminal hair. In our subjects with alopecia who had a continuous distribution of the rate of hair growth, the normal hair cycle is disrupted and the change from anagen to catagen reduced. It is not known whether individual hairs show a constant rate of growth in the anagen period even though growth rates differ in each hair. To establish this it would be necessary to measure the growth rate of the same hair throughout an entire cycle.

We consider that our method is of use in measuring the rate of hair growth and to establish the number of hairs per unit area, the hair diameter and the groupings. The method is a rapid one and could be useful in the diagnosis of different types of alopecia and in the objective assessment of agents used to promote regrowth of hair.

References

1 Saito M, Uzuka M, Sakamoto H, Kobori T. Hair growth. In. *Advances in Biology of Skin* (Montagna W, Dobson RL, eds). Oxford. Pergamon Press, 1969; 153–60.
2 Harkness DR, Bern HA. Radioautographic studies of hair growth in the mouse. *Acta Anatomica* 1957; 31: 35–9.
3 Matias JR, Alani R, Orentreich N. Measurement of the rate of hair growth using a fluorescent tracer technique. *J Soc Cosmet Chem* 1987; 38: 77–85.
4 Barman JM, Astore I, Pecoraro V. Method, technique and computation in the study of the trophic state of human scalp hair. *J Invest Dermatol* 1964; 42: 421–5.
5 Barman JM, Astore I, Pecoraro V. The normal trichogram of the adult. *J Invest Dermatol* 1965; 44: 233–6.
6 Rushton H, James KC, Mortimer CH. The unit area trichogram in the assessment of androgen-dependent alopecia. *Br J Dermatol* 1983; 109: 429–37.
7 Takashima I, Kawagishi I. Comparative study of hair growth in mammals, with special references to hair grouping and hair cycle, and hair growth rate in the juvenile stumptailed macaque. In: *Biology and Disease of the Hair* (Kobori T, Montagna W, eds), Tokyo: University Tokyo Press, 1976; 457–71.
8 Saitoh M, Uzuka M, Sakamoto M. Human hair cycle. *J Invest Dermatol* 1970; 54: 65–81.

9 Guarrera M, Ciulla MP. A quantitative evaluation of hair loss: the phototrichogram. *J Appl Cosmetol* 1986; 4: 61–6.

10 Friedel J, Will F, Grosshans E. Phototrichogram. Adaptation, standardization and application. *Ann Dermatol Venereol* 1989; 116: 629–36.

11 Fiquet C, Courtois M. Une technique originale d'appréciation de la croissance et de la chute des cheveux. *Cutis* 1979; 3: 975–83.

12 Pelfini C, Calligaro A. Evaluation of hair growth by means of morphometric computerized analysis. *J Appl Cosmetol* 1986; 4: 67–76.

13 Pelfini C, Fideli D, Speziali A, Vignini M. Effects of a topical preparation on some hair growth parameters, evaluated utilizing a morphometric computerized analysis. *Int J Cosmet Sci* 1987; 9: 1–11.

British Journal of Dermatology (1991) 125, 130–135. ADONIS 000709639100182V

Decreased sweat-gland number and function in patients with alopecia areata

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Accepted for publication 8 March 1991

Summary In this study the number and function of sweat glands on the dorsum of the hand and foot were evaluated using a silastic imprint method. Twenty patients, 11 females and nine males with either alopecia areata, alopecia totalis, or alopecia universalis were included in the study. Patients showed a significant decrease in activated sweat-gland number and function compared to matched controls ($P < 0{\cdot}001$). There was no significant relationship between the degree of sweat-gland dysfunction and extent of hair loss, concurrent illnesses, or treatment. These findings suggest that eccrine sweat-gland function is compromised in patients with alopecia areata.

The aetiology of alopecia areata (AA) is unknown although most investigators believe this disease is mediated by the immune system.[1–3] The hair and nails are the principal ectodermal-derived appendages involved in AA. Unlike the group of disorders known as the ectodermal dysplasias, there has been no evidence to suggest that in addition to hair and nails, the teeth or sweat glands are also affected in patients with AA. In this study sweat-gland function was investigated in 20 patients with alopecia areata.

Methods

Disease classification

Based on the extent of their disease, patients were classified into one of the following three groups: patchy alopecia areata (AA), alopecia totalis (AT), and alopecia universalis (AU). Patients with reticular and ophiasis pattern AA were classified as having patchy AA. Patients with diffuse hair loss involving primarily the scalp were classified as having AT, and those with complete body hair loss as AU. The diagnosis of AA was confirmed with histopathological examination of scalp biopsy specimens in all patients except three, a 25-year-old female with AA affecting the eyelashes and two 3-year-old children with patchy AA.

Subjects

Twenty patients, 11 females and nine males between the ages of 3 and 69 years, participated in this study. Six patients (three females and three males) had AA, five (four females and one male) had AT, and nine (four females and five males) had AU. Control subjects were matched for sex, and were within 5% of the patients' age, weight and height (Table 1).

Two patients had a history of autoimmune diseases; one had Grave's disease (Patient 5), the other vitiligo (Patient 13). Other concurrent illnesses reported by the patients at the time of the study included hypothyroidism (two patients), depression (two patients) and hypoparathyroidism (one patient).

Fourteen patients were not receiving any treatment for their AA at the time they participated in this study. Six patients were applying topical preparations, and one patient was taking oral prednisone, as well as applying a topical steroid ointment. The specific medications used are shown in Table 1.

Sweat-gland testing

Eccrine sweat-gland number and function were evaluated by the silastic-imprint method. This technique provides an easily reproducible and non-invasive testing method, which when used in combination with computerized image processing, results in an extremely sensitive method for the quantitative evaluation of sweat-gland number and function.[4,5] Sweating was stimulated on the dorsum of the hand and foot (over the first intermetacarpal and first intermetatarsal space, respectively) by iontophoresis of 1% aqueous pilocarpine nitrate using 2 mA direct current for 5 min. After stimulation, each area was wiped clean of excess pilocarpine, and 5

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Table 1. Patient demographic data

Patient number	Age (years)	Sex	Type of alopecia areata	Concurrent illness	Alopecia areata treatment at time of testing		
					Medication	Dosage	Duration of use
1	3	M	Patchy	None	None		
2	3	M	Patchy	None	Valisone 0·1% lotion	1 ml b.i.d.	1 month
3	18	F	Patchy	None	Topical 2·0% minoxidil	1 ml b.i.d.	2 years
4	25	F	Patchy	Hypothyroidism	None		
5	36	M	Patchy	Grave's disease	None		
6	37	F	Patchy	Depression	None		
7	5	F	Totalis	None	Diprolene lotion	1 ml b.i.d.	1 week
8	15	F	Totalis	None	None		
9	26	M	Totalis	None	None		
10	27	F	Totalis	None	None		
11	69	F	Totalis	Hypothyroidism	Rogaine	1 ml b.i.d.	14 months
					Retin A 0·05% lotion	2 ml q.o.d.	6 months
					Diprolene lotion	1 ml b.i.d.	14 months
12	9	F	Universalis	None	None		
13	9	M	Universalis	Vitiligo	None		
14	26	M	Universalis	Hypoparathyroidism	None		
15	26	M	Universalis	None	None		
16	27	M	Universalis	None	Anthralin 0·5%	b.i.d.	1 week
17	30	F	Universalis	Depression	None		
18	37	F	Universalis	None	None		
19	43	F	Universalis	None	Prednisone	15 mg p.o. q.d.	3 months
					Lidex 0·05% ointment	1 ml q.d.	3 months
20	43	M	Universalis	None	None		

ml of liquid silastic mixed with hardener (Kerr Co., Romulus, MI, U.S.A.) was immediately spread with a spatula over the stimulated site. The moulds hardened in 4 min and were then peeled from the skin and mounted on microscopic slides.

Ambient temperature and humidity are not known to effect the sweat production caused by pilocarpine stimulation. Because pilocarpine bypasses preganglionic neurones causing direct stimulation of the postganglionic receptors, and because the concentration of pilocarpine used in our study is thought to completely saturate the cholinergic receptor sites causing maximal sweat secretion, it is unlikely that minor temperature or humidity changes would alter the pilocarpine stimulation test. However, to avoid this source of variability, we did control temperature by testing all patients in the dermatology clinic where ambient temperature varies from 21·5 to 24·0°C.

Measurements

Sweat droplets emerging on to the skin surface from stimulated sweat ducts made impressions which were retained in the hardening silastic moulds. The size of each impression was proportional to the volume of output of the gland, and was therefore an index of activated sweat-gland function.[4,5] The moulds were placed on microscope slides and analysed using computerized image processing (International Imaging Systems). An image of the silastic mould was projected through a microscope, and processed using a software system that enhanced contrast (Fig. 1). In the silastic mould, skin lines appear as elevations and sweat droplets as indentations. During the analysis, all skin lines were filtered out. Once filtration was complete, sweat-droplet density per cm^2 and mean sweat-droplet size were calculated and the total area occupied by sweat droplets in the 1-cm^2 area was measured in pixels. Because it is a fixed function of volume, area of droplets was used as a proxy for sweat volume. The per cent difference between each patient and control was calculated for sweat-droplet density, sweat-droplet size, and total area occupied by sweat droplets. If the patient's value was greater than the control's, the per cent difference was noted with a minus sign.

Computerized analysis of sweat-droplet density correlated well with manual counts performed by counting the number of sweat droplets in the moulds under the light microscope ($R = 0{\cdot}993$). Reproducibility of computerized measurements was assessed on three separate occasions and the coefficient of variability was found to be less than 0·012 (data not shown).

Statistical analysis

Results from patients and controls were compared using Student's *t*-test for paired observations. One-way ANOVA of the per cent differences between patients and controls was used to compare the degree of dysfunction in the three groups of patients (patchy vs. totalis vs. universalis). This same method was also used to compare sweat-gland number and function in treated vs. untreated patients, and patients with concurrent illnesses vs. patients without concurrent disease.

Results

All patients completed testing at both the hand and foot sites except for two who experienced discomfort during the testing procedure. A 9-year-old female (Patient 12) with a well-known history of syncopal episodes associated with venopuncture and other medical procedures, experienced the same during the sweat-gland stimulation procedure. Data was only obtained from her hand site before testing had to be discontinued. A 5-year-old female (Patient 7) stated she felt discomfort during testing of her foot site and objected to further testing on her hand.

Values obtained for activated sweat-droplet density, mean sweat-droplet size, the total area occupied by sweat droplets in a 1 cm^2 area, and the difference between the patient and control for each of these values are shown in Table 2. The *t*-test analysis for paired observations demonstrated that patients with AA had significantly decreased sweat-droplet densities, mean sweat-droplet size, and total area occupied by sweat droplets in 1 cm^2 as compared to their respective controls ($P < 0{\cdot}001$) (Table 3). The patients showed an average decrease in the number of activated sweat glands on their hand and foot sites of 42·5 and 38·7%, respectively. Sweat-gland function, as measured by mean sweat-droplet size, was also decreased in the AA patients as compared to the controls. The average decrease at the hand site was 41·6% and 37·9% at the foot site (Table 3). No relationship between any of the sweat-gland parameters studied and disease extent, concurrent illness, or treatment was found (Table 4).

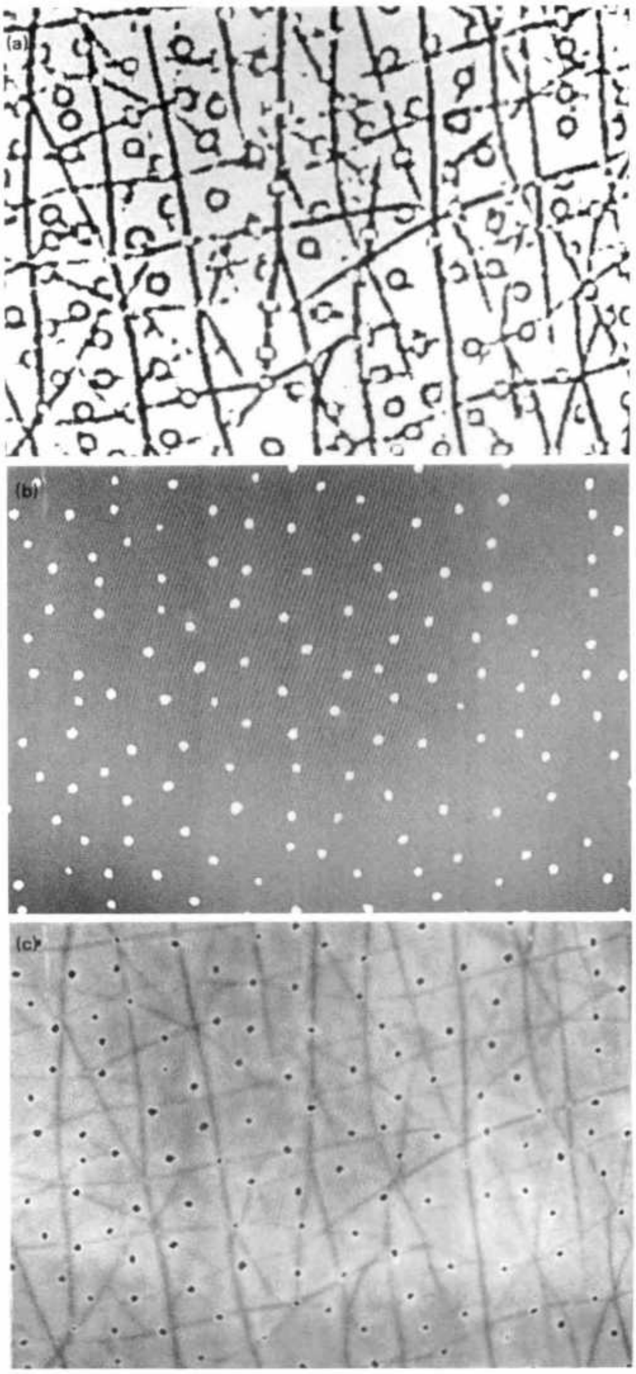

Figure 1. (a) Computerized image of a silastic mould on the video screen prior to filtration of skin-line artefact. (b) Same image after filtration. (c) Computerized image superimposed on direct image of silastic mould.

Condensing the data in Table 2 by giving the mean and SD for each of the three alopecia types is not appropriate, because sweat-gland density appears to be related to age and body size. Although there is controversy over this topic, our patient population tended to

Table 2. Comparison of sweat-gland number and function in patients vs. controls

	Patient/ control matched pair	Age (years)	Sex	Type of alopecia areata	Sweat-droplet density (SDD)		Sweat-droplet size		Total area occupied by sweat droplets	
					Hand (SD/cm^2)	Foot (SD/cm^2)	Hand (pixels)	Foot (pixels)	Hand (pixels/cm^2)	Foot (pixels/cm^2)
Patient	1	3	M	Patchy	624 (−5)*†	464 (23)	11·20 (71)	12·51 (39)	9239 (36)	7679 (71)
Control		3			593	573	19·20	17·35	15057	13136
Patient	2	3	M	Patchy	428 (39)	325 (76)	18·79 (2)	21·60 (−20)	10634 (42)	9290 (41)
Control		3			593	573	19·20	17·35	15057	13136
Patient	3	18	F	Patchy	349 (7)	228 (28)	10·57 (62)	10·26 (43)	4885 (74)	3087 (82)
Control		18			374	290	17·16	14·67	8495	5632
Patient	4	25	F	Patchy	432 (−2)	275 (−11)	17·30 (16)	17·79 (25)	9876 (14)	6458 (12)
Control		25			424	245	20·00	22·31	11220	7229
Patient	5	36	M	Patchy	259 (29)	197 (17)	24·76 (−7)	19·88 (45)	8492 (29)	5188 (68)
Control		36			361	230	22·96	28·73	10954	8735
Patient	6	37	F	Patchy	287 (19)	215 (6)	19·24 (46)	17·63 (73)	7312 (73)	5007 (82)
Control		36			341	227	28·00	30·43	12630	9130
Patient	7	5	F	Totalis	ND	339 (11)	ND	10·72 (57)	ND	4804 (74)
Control		5				374		16·85		8344
Patient	8	15	F	Totalis	231 (98)	173 (98)	23·94 (−18)	15·39 (−0·1)	7303 (63)	3524 (48)
Control		14			456	256	19·68	15·38	11866	5214
Patient	9	26	M	Totalis	216 (6)	170 (20)	31·17 (−13)	22·38 (36)	8886 (−0·8)	5036 (62)
Control		27			228	203	27·15	30·32	8173	8157
Patient	10	27	F	Totalis	321 (50)	232 (15)	13·45 (74)	13·13 (55)	5716 (161)	4032 (78)
Control		28			482	267	23·39	20·31	14898	7171
Patient	11	69	F	Totalis	272 (−18)	107 (79)	18·59 (34)	19·51 (47)	6692 (11)	2751 (164)
Control		69			225	191	24·99	28·70	7422	7261
Patient	12	9	F	Universalis	293 (57)	ND	9·39 (102)	ND	3642 (217)	ND
Control		9			461		18·98		11561	
Patient	13	9	M	Universalis	172 (176)	232 (44)	8·54 (156)	19·87 (21)	1939 (606)	6121 (13)
Control		9			474	336	21·83	15·64	13690	6943
Patient	14	26	M	Universalis	189 (20)	188 (8)	11·69 (132)	20·10 (50)	2922 (180)	5005 (63)
Control		27			228	203	27·15	30·23	8173	8157
Patient	15	26	M	Universalis	210 (33)	207 (7)	24·18 (18)	30·67 (19)	6697 (57)	8372 (28)
Control		25			279	222	28·49	36·63	10512	10732
Patient	16	27	M	Universalis	178 (78)	198 (47)	19·48 (54)	19·15 (53)	4577 (174)	5016 (126)
Control		28			316	291	30·30	29·31	12552	11312
Patient	17	30	F	Universalis	297 (27)	142 (57)	20·63 (23)	23·51 (23)	8088 (55)	4420 (94)
Control		31			375	224	25·30	28·95	12547	8570
Patient	18	37	F	Universalis	253 (78)	107 (214)	17·88 (8)	15·20 (16)	5973 (93)	2158 (267)
Control		35			451	338	19·31	17·69	11506	7908
Patient	19	43	F	Universalis	225 (28)	228 (25)	16·76 (19)	19·46 (39)	4978 (52)	5856 (73)
Control		44			288	285	19·90	26·97	7582	10142
Patient	20	43	M	Universalis	173 (78)	220 (21)	20·93 (11)	16·96 (59)	4792 (98)	4935 (93)
Control		41			309	267	23·24	26·94	9482	10861

ND, not done.

* ( ) = % difference of patients and controls.

† −denotes a percentage difference where patient values are greater than control values.

Table 3. Mean per cent difference in patient/control pairs and *t*-test analysis for paired observations

	Sweat-droplet density (sweat droplets/cm^2)		Mean sweat-droplet size (pixels)		Total area occupied by sweat droplets (pixels)	
	Hand	Foot	Hand	Foot	Hand	Foot
Mean difference	97	72	5·1	5·7	4775·5	3561·6
Standard error	21·2	15·8	1·24	1·06	682·4	345·7
Mean per cent difference	42·5	38·7	41·6	37·9	106·6	81·0
P-value	<0·0005	<0·0005	<0·001	<0·0005	<0·0005	<0·0005

Table 4. Results of one-way ANOVA, comparing degree of sweat-gland dysfunction between patient groups

	P-values					
	Sweat-droplet density		Mean sweat-droplet size		Total area occupied by sweat droplets	
Groups compared	Hand	Foot	Hand	Foot	Hand	Foot
Patchy vs totalis vs universalis	0·12	0·54	0·36	0·94	0·15	0·55
Treated vs. untreated	0·40	0·74	0·70	0·93	0·51	0·54
Concurrent disease vs. no concurrent disease	0·69	0·49	0·50	0·81	0·43	0·45

show that the sweat-gland density was greater in children and decreased with age and body size. We selected controls who were sex-matched and within 5% of the patients' age, weight and height to eliminate these variables. Because of the large variation in patient age and body size it was necessary to present the results of paired testing for each patient and control rather than combining each group and calculating a mean and standard deviation.

Discussion

We have demonstrated a significant decrease in activated sweat-gland number and function in patients with alopecia areata compared to matched controls. The degree of sweat-gland dysfunction was not related to the extent of AA, therapy or concurrent diseases. The dysfunction should be at the level of the sweat gland, the sudomotor axon or its ganglion, or be postganglionic.

The eccrine sweat gland is a cutaneous appendage that plays a major role in thermoregulation. Its development begins during the third foetal month and is thought to be complete prior to birth with no new glands being added with age and growth.[6,7] Children, with their smaller surface area would therefore be expected to have a greater density of sweat glands than adults.[8] However, a study by Jorgenson *et al.* did not support the expectation that newborns would have the greatest density of sweat glands, children the next greatest, and adults the least.[9] There is also controversy in the literature as regards the relationship between sweat-gland number and function, and sex and race.[6,10–12]

The eccrine sweat gland is innervated by non-myelinated class C sympathetic postganglionic fibres.[13] The principal neurotransmitter is acetylcholine, but adrenergic transmitters have been demonstrated to have minor activity.[14,15] Sweat production can be stimulated by α-adrenergic, β-adrenergic, and cholinergic agonists, although the maximal secretory rate differs markedly between the three, with the ratio in humans being 1:1:5, respectively.[16] Iontophoresis of the cholinergic agonist pilocarpine is believed to stimulate eccrine glands directly, thereby testing the postganglionic innervation in conjunction with the function of the sweat gland itself. Central nervous system and preganglionic influences are not known to affect test results obtained with pilocarpine stimulation.[5,17] Consequently, neuronal dysfunction proximal to the postganglionic structures would be unlikely to explain the decrease in sweat-gland number and function shown in our study.

Long-term studies may provide information as to whether sweat-gland function returns to normal after the alopecia areata process resolves, or whether abnormalities of activated sweat-gland number and function persist regardless of the degree of hair loss.

Acknowledgments

This work was supported in part by a grant from the Minnesota Medical Foundation. We thank Dr Tim Church for his assistance with the statistical analyses.

References

1 Alopecia areata–an autoimmune disease? Editorial. *Lancet* 1984; **i:** 1335–6.

2 Mitchell AJ, Krull E. Alopecia areata: pathogenesis and treatment. *J Am Acad Dermatol* 1984; **11:** 763–5.

3 Hordinsky MK, Kalish RS. Autoreactive cells in alopecia areata. *J Invest Dermatol* 1991; (in press).

4 Kennedy W, Sakuta M, Sutherland D, Goetz FC. Quantitation of the sweating deficiency in diabetes mellitus. *Ann Neurol* 1984; **15:** 482–8.

5 Kennedy WR, Navarro X. Sympathetic sudomotor function in diabetic neuropathy. *Arch Neurol* 1989; **46:** 1182–6.

6 Kuno Y. *Human Perspiration.* Springfield, Illinois: Charles C. Thomas, 1956; 42–93.

7 Montagna W, Parakkal PF. *The Structure and Function of the Skin.* 3rd edn. New York: Academic Press, 1974; 376–96.

8 Frias JL, Smith DW. Diminished sweat pores in hypohidrotic ectodermal dysplasia: A new method for assessment. *J Pediatr* 1968; **72:** 606–10.

9 Jorgenson RJ, Salinas CF, Dowben JS, St John DL. A population study on the density of palmar sweat pores. *Birth Defects* 1988; **24:** 51–63.

10 Kral JA, Zenisek A, Kopecka J. Crossed locomotor and subomotor innervation: asymmetry and sex differences in sweat gland irritability. *Int J Physiol* 1969; **27:** 165–70.

11 Juniper K, Dykman RA. Skin resistance, sweat gland counts, salivary flow, and gastric secretion: age, race, and sex differences, and intercorrelations. *Psychophysiology* 1967; **4:** 216–22.

12 Thompson ML. A comparison between the number and distribution of functioning eccrine sweat glands in Europeans and Africans. *J Physiol* 1954; **123:** 225–33.

13 Sato K, Kang WH, Saga K, Sata KT. Biology of sweat glands and their disorders. I: Normal sweat gland function. *J Am Acad Dermatol* 1989; **20:** 537–62.

14 Uno H. Sympathetic innervation of the sweat glands and piloarrector muscle of macaques and human beings. *J Invest Dermatol* 1977; **69:** 112–30.

15 Uno H, Montagna W. Catecholamine-containing nerve terminals of the eccrine sweat glands of macaques. *Cell Tissue Res* 1975; **158:** 1–13.

16 Sato K, Sato F. Defective beta adrenergic response of cystic fibrosis sweat glands *in vivo* and *in vitro*. *J Clin Invest* 1984; **73:** 1763–71.

17 Cardone C, Dyck PJ. A neuropathic deficit, decreased sweating, is prevented and ameliorated by euglycemia in streptozocin diabetes in rats. *J Clin Invest* 1990; **86:** 248–53.

British Journal of Dermatology (1991) 125, 136–139. ADONIS 000709639100183A

Phenytoin has little effect on *in-vitro* models of wound healing

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Accepted for publication 8 March 1991

Summary It has been reported that phenytoin induces gingival and connective tissue hyperplasia and may be of use in wound healing. In this study the effect of phenytoin on human epidermal keratinocytes and skin fibroblasts has been investigated *in vitro*. Cell cultures were exposed to increasing concentrations of phenytoin from 10^{-9} to 10^{-4} M in the presence of 1 and 10% serum supplemented medium. In addition the effect of phenytoin on epidermal cell migration (epiboly) has been investigated using organ culture of human skin. No stimulation of cell growth was observed, and only a mild toxicity affecting keratinocytes was seen at the highest concentrations. Similarly, no effect on epidermal cell migration *in vitro* was observed. The lack of a direct effect *in vitro* suggests that any *in-vivo* effect was not the result of interaction between phenytoin and keratinocytes or fibroblasts but possibly due to indirect modulation via other cell types, such as inflammatory or lymphoreticular cells.

Phenytoin (sodium diphenylhydantoin), a well-known anticonvulsant, anti-arrythmic and antineuralgic drug, has been in clinical use for over 50 years. Its potential as a therapeutic agent in wound healing was suggested by Bodkin in 1945.[1] Subsequently Shapiro[2] and other workers[3,4] demonstrated the apparent beneficial effects of phenytoin in the healing of periodontal disease. Studies with animal and human gingival fibroblasts and the apparent induction of gingival hyperplasia in epileptics have also indicated the positive effects of phenytoin.[5–9]

The drug's potential use in dermatology and cutaneous wound healing has been emphasized by the apparently successful treatment of recessive dystrophic epidermolysis bullosa, a disorder with elevated levels of collagenase.[10] Phenytoin also suppresses the synthesis and/or secretion of collagenase in human fibroblasts and skin explants.[10,11]

Evidence for the positive wound-healing effects of phenytoin has also been reported.[12,13] Initial assessment suggested that phenytoin applied to cutaneous ulcers, burns and other wound surfaces significantly decreased pain, wound exudate and bacterial contamination and accelerated wound healing. Histological examination of phenytoin-treated wounds showed evidence of increased numbers of fibroblasts, increased collagen content, accelerated epidermal migration and angiogenesis. However, controlled *in-vitro* studies have not been performed, and the aim of the present study was to examine objectively the effect of phenytoin on cultured human skin fibroblasts and keratinocytes *in vitro*. The effect of the drug on epidermal cell migration was also studied under similar experimental conditions.

Methods

Fibroblasts and keratinocytes

Human dermal fibroblast and epidermal keratinocyte cultures were established using previously described methods.[14] Fibroblasts were obtained from dermal fragments derived from the circumcised foreskin of a 5-year-old Caucasian male. Subpassage was performed at confluency and fourth-passage fibroblasts were used for the experiment.

Human epidermal keratinocytes from the circumcised foreskin of another 5-year-old Caucasian male were grown in a mixture of DMEM: Ham's F12 (3:1) containing 10% fetal calf serum, 0·4 μg/ml hydrocortisone, 5 μg/ml insulin, 10 ng/ml cholera toxin, 5 μg/ml transferrin and 2×10^{-11} M triiodothyronine (HK medium). During the first 10 days of primary culture the medium also contained penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were allowed to grow till confluency, with media changes every third day.

Preparation of phenytoin

Powdered phenytoin (sodium diphenylhydantoin, Parke

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Paper presented at British Association of Dermatologists 70th Annual Meeting, University of Warwick, 18–21 July 1990.

Davis and Co., batch No. 110-14420), was dissolved at room temperature in absolute alcohol to give a stock solution containing 25 mg/ml of the drug. The freshly prepared stock solution was then diluted in DMEM containing 10 or 1% serum to give a starting concentration of 10^{-4} M of phenytoin. This was then diluted with the appropriate media to give a range of concentrations of 10^{-5}–10^{-9} M for the experiments. The control solution containing only solvent, without phenytoin, contained the same amount of absolute alcohol as was present in the media containing 10^{-4} M phenytoin.

Effect of phenytoin on human skin fibroblast proliferation

Fourth-passage fibroblasts were cultured in 26 × 36 mm rectangular plastic culture wells (LuxR) in DMEM with 10% newborn calf serum (NBCS). Each well contained 6×10^{-4} fibroblasts in 2 ml of media. After 24 h the media was removed and replaced with media containing phenytoin in concentrations of 10^{-9}–10^{-4} M with control wells containing DMEM and 10% NBCS only or DMEM and 10% NBCS plus alcohol.

An identical set of cultures was set up using DMEM with only 1% NBCS instead of 10% NBCS to create a relatively compromised environment for the cells.

After 48 h of incubation at 37°C in a 5% CO_2 humidified atmosphere, the cells were washed in Dulbecco's salt solution (DSS) and fixed *in situ* with 10% phosphate-buffered formalin and then stained with 0·1% crystal violet. After thorough washing with distilled water the cells were air-dried and then the percentage-area fraction of the stained cells was assessed in each dish using a computerized image-analysis system (Optomax V). Six sets of experiments were performed using the same passage of fibroblasts under identical conditions.

Effect of phenytoin on human epidermal keratinocyte proliferation

At confluency, the primary keratinocyte cultures were treated with EDTA and trypsin and subsequently subcultured on to collagen-coated 35-mm-diameter plastic culture dishes (Nunclon R). Initially, 2 ml of HK medium, containing $3{\cdot}5 \times 10^5$ cells, was put into each dish and left to incubate for 24 h. The media was then removed and HK medium containing phenytoin in concentrations of 10^{-9}–10^{-4} M was added, with similar controls to those used in the fibroblast experiments. In our pilot studies it was found that the keratinocytes were more sensitive to the alcohol vehicle than the fibroblasts. To eliminate spurious inhibition of proliferation in the media containing more alcohol, the alcohol concentration was standardized in all the sets to be equivalent to the amount present in the media containing 10^{-4} M phenytoin.

Again, two sets of experiments were set up, one using HK medium with 10% fetal calf serum (FCS) and the other with medium containing 1% FCS. The cultures were then incubated for a further 5 days and on the sixth day the cells were washed in DSS, fixed *in situ* with 10% buffered formalin and stained with 0·1% crystal violet in the same way as described for the fibroblast cultures. The percentage-area fraction of the stained colonies was then assessed using the image-analysis method described earlier. The experiments were all performed in triplicate using the same strain of keratinocytes.

Effect of phenytoin on epidermal cell migration (epiboly)

The effect of phenytoin on epidermal cell migration was studied using human skin explants derived from three normal Caucasian adults. These explants were from thigh skin of a 44-year-old male (epiboly I), abdominal skin of a 39-year-old male (epiboly II), and breast skin of a 58-year-old female (epiboly III). The samples were all obtained from discarded surgical specimens of normal skin.

In each experiment, as much of the dermis as possible was trimmed off and the skin cut as atraumatically as possible into 2-mm-square pieces. These were washed several times in DSS and then placed in 5-cm-diameter plastic culture dishes containing DMEM with either 10% FCS or 1% FCS, and phenytoin was added in concentrations of 10^{-9}–10^{-4} M as described earlier. In addition, 100 U/ml of penicillin and 100 μg/ml of streptomycin were added to each dish.

Between 10 and 20 explants were put into each dish and left to incubate at 37°C in a 5% CO_2 humidified atmosphere for 48 h. The explants were then washed in DSS and fixed in 10% buffered formalin, paraffin-blocked, sectioned and stained with haematoxylin and eosin.

The degree of epiboly, i.e. the migration of epidermal cells beyond the severed edges of the epidermis, was assessed in terms of the area of the migrating sheets using a computerized image analysis system (Optomax V).

Results

Effect of phenytoin on skin fibroblast proliferation

Exposure of cultured human skin fibroblasts to varying

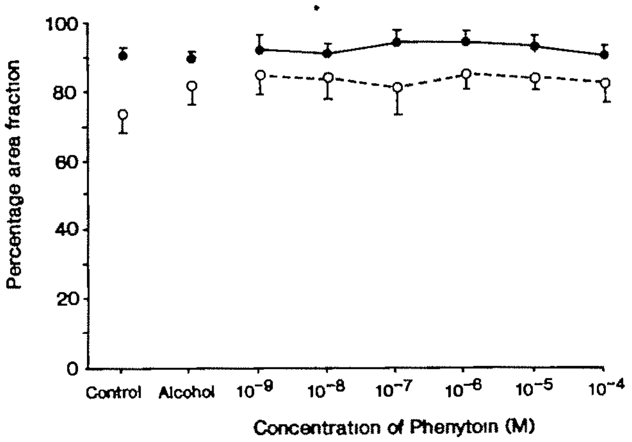

Figure 1. The effect of phenytoin on human skin fibroblast proliferation in medium containing 10% newborn calf serum (●) or 1% newborn calf serum (○). Results are expressed as percentage-area fraction of dish covered by cells.

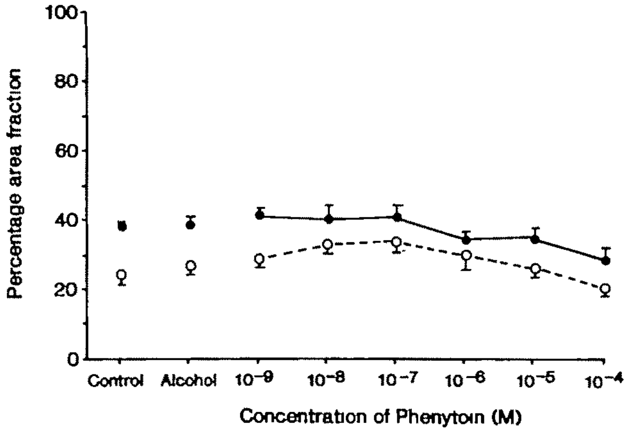

Figure 2. The effect of phenytoin on human epidermal keratinocyte proliferation in medium containing 10% fetal calf serum (●) or 1% fetal calf serum (○). Results are expressed as percentage-area fraction of dish covered by cells.

concentrations of phenytoin did not result in any significant stimulation of growth in medium with either 10% or 1% NBCS (Fig. 1).

Effect of phenytoin on human epidermal keratinocyte proliferation

The effect of phenytoin on the human keratinocyte cultures is shown in Figure 2. Once again, no significant stimulation of proliferation was noted. However, there was a mild but definite toxic effect noted in both sets of media conditions (i.e. 10% FCS and 1% FCS) with a phenytoin concentration of 10^{-4} M.

Table 1. Effect of phenytoin on percentage area of control of epiboly (10% FCS)

Phenytoin concentration (M)	Percentage area of control Thigh/M/44 Epiboly I	 Abdomen/M/39 Epiboly II	 Breast/F/58 Epiboly III	Average
Alcohol	80·3	126·8	103 1	103·4
10^{-9} M	169·2	131·8	87·0	129·3
10^{-8} M	141·2	171·0	62·3	124·8
10^{-7} M	154·5	119·2	82·3	118·7
10^{-6} M	190 0	181·5	105·3	158·9
10^{-5} M	192·4	120·9	107·8	140·4
10^{-4} M	144·3	96·7	87·2	109·4

Effect of phenytoin on epidermal cell migration (epiboly)

The epiboly phenomenon was studied in three types of normal adult human skin: thigh skin (epiboly I), abdominal skin (epiboly II) and breast skin (epiboly III). The result of the experiments using 10% FCS are shown in Table 1, where the average areas of the migrating limbs are expressed as a percentage of the control containing only media and serum. In the more optimal medium containing 10% FCS there was an apparent stimulation of epiboly, particularly at a concentration of phenytoin at 10^{-6} M, when the average percentages of all three sets of experiments were assessed. However, one-way analysis of variance between the values in the different groups failed to give a statistically significant result. Similar results were obtained with the 1% FCS experiments (results not shown).

Discussion

The suggestion that topical phenytoin may be of value in treating cutaneous wounds is attractive as it would mean the ready availability of a well-known, inexpensive and easily applied drug for problems which incur a great deal of time, expense and suffering for patients. Initial investigations *in vitro* were conducted with gingival fibroblasts and connective tissue. Later studies with cutaneous fibroblasts gave apparently contradictory results. Thus it was shown that a biphasic response existed, with stimulation of growth occurring in the first 3 h of exposure followed by inhibition with further exposure to the drug.[10,11] In contrast, no effect on collagen lattice contraction by fibroblasts, a model of skin wound contraction, could be demonstrated.[15] Studies on human gingival fibroblasts suggested that the effect of the drug occurred only in certain groups of genetically predetermined and stable subpopulations of phenytoin-sensitive resident gingival fibroblasts.[9]

In our study there was no effect on dermal fibroblast proliferation after 48 h of incubation with phenytoin. Similarly there was no significant stimulation of keratinocyte growth, although there was a mild cytotoxic effect seen at the highest concentration of phenytoin that was tested, i.e. 10^{-4} M. No effect was seen in the *in-vitro* model of wound healing using explants of whole skin.

The range of concentrations tested *in vitro* cover the serum phenytoin concentrations normally seen in patients ($3–5 \times 10^{-5}$ M).[16] When applied topically, higher concentrations may be achieved. However, the evidence from keratinocytes suggests that if concentrations higher than 10^{-4} M were reached, a cytotoxic rather than a stimulatory effect occurred. Although only a limited number of cell lines have been examined, the negative findings over a wide range of concentrations in three separate systems and different serum concentrations strongly suggest that phenytoin does not have stimulatory properties.

It could be postulated that phenytoin might be acting indirectly *in vivo* on keratinocytes and fibroblasts via stimulation of inflammatory cell cytokine production. This would explain the lack of a direct effect of the drug in the 'isolated' *in-vitro* cell-culture systems examined in our study. This postulated mode of action would also help to explain the drug's apparent antibacterial effect when topically applied *in vivo* but not *in vitro*.[12] Phenytoin acts on cation transport across membranes and has been shown to increase intracellular potassium and block sodium and calcium influx under various conditions.[17] Much of cytokine and growth-factor activity is dependent on changes in intracellular cation levels, particularly of calcium. Further support for this hypothesis is the occurrence of gingival hyperplasia in some patients taking the calcium-channel block Nifedipine.[18]

Even previously observed direct beneficial effects on matrix contents and collagen production by cultured fibroblasts have been mainly noted in fibroblasts which have been derived from inflamed gingival tissues, suggesting that some kind of priming of these cells and the matrix components has occurred under the influence of chronic inflammation. The erratic responses seen in various studies have led to the suggestion that the peculiar behaviour of these cells in culture may be a reflection of the state of health or disease in the tissue of origin.[8] This may explain the lack of response in the fibroblast and keratinocyte experiments, as these cells were derived from healthy juvenile foreskins.

In conclusion, the lack of any consistent effect of phenytoin found in these *in-vitro* studies raises further doubts as to whether this compound has any substantial effect on the wound-healing process.

References

1 Bodkin LG. Oral therapy for pruritus ani. *Am J Dig Dis* 1945; **12:** 255–7.

2 Shapiro M. Acceleration of gingival wound healing in non-epileptic patients receiving diphenylhydantoin sodium. *Exp Med Surg* 1958; **16:** 41–53.

3 Otto G, Ludewig R, Kotz JH. Specific action of local phenytoin application on periodontal disease. *Stomatol DDR* 1977; **98:** 1–8.

4 Payan J. A study of changes in the gum during treatment with diphenylhydantoin sodium. *Rev Odontol Stomatol* 1972; **19:** 47–53.

5 Shafer WG. Effect of Dilantin sodium on growth of human fibroblast-like cell cultures. *Proc Soc Exp Biol* 1960; **104:** 198–201.

6 Hassel TM, Page RC, Narayanan AS, Cooper CG. Diphenylhydantoin (Dilantin) gingival hyperplasia: drug-induced abnormality of connective tissue. *Proc Natl Acad Sci USA* 1976; **73:** 2909–12.

7 Hassel TM, Gilbert GH. Phenytoin sensitivity of fibroblasts as the basis of susceptibility to gingival enlargement. *Am J Pathol* 1983; **112:** 218–23.

8 Kantor ML, Hassel TM. Increased accumulation of sulphated glycoaminoglycans in cultures of human fibroblasts from phenytoin-induced gingival overgrowth. *J Dent Res* 1983; **62:** 383–7.

9 Narayanan AS, Meyers DF, Page RC. Regulation of collagen production in fibroblasts cultured from normal and phenytoin-induced hyperplastic human gingiva. *J Periodontal Res* 1988; **23:** 118–21.

10 Bauer EA, Cooper TW, Tucker DR, Esterly NB. Phenytoin therapy for recessive dystrophic epidermolysis bullosa–clinical trial and proposed mechanisms of action on collagenase. *New Engl J Med* 1980; **303:** 776–81.

11 Moy LS, Tan EML, Holness R, Uitto J. Phenytoin modulates connective tissue metabolism and cell proliferation in human skin fibroblast cultures. *Arch Dermatol* 1985; **121:** 79–83.

12 Smith BH, Moore M, Jain K. Report on the first international conference on the uses of phenytoin in dermatology. *Int J Dermatol* 1988; **27:** 528–30.

13 Madegegh S, Salehian B, Tavassoli M *et al.* Uses of phenytoin in healing war and non-war wounds. *Int J Dermatol* 1989; **28:** 347–50.

14 Dykes PJ, Brunt J, Marks R. The effect of cyclosporin on human epidermal keratinocytes *in vitro*. *Br J Dermatol* 1990; **122:** 173–80.

15 Adams LW, Priestley GC. Contraction of collagen lattices by skin fibroblasts: drug induced changes. *Arch Dermatol Res* 1988; **280:** 114–8.

16 Kilpatrick CJ, Wanawimolruk S, Wing LMH. Plasma concentration of unbound phenytoin in the management of epilepsy. *Br J Clin Pharmacol* 1984; **17:** 539–46.

17 Finkel MJ. Phenytoin revisited. *Clinical Therapeutics* 1984; **6:** 577–91.

18 Lucas RM, Howell LP, Wall BA. Nifedipine-induced gingival hyperplasia. *J Periodontol* 1985; **56:** 211–5.

British Journal of Dermatology (1991) 125, 140–146. ADONIS 000709639100184N

Skin susceptibility in uninvolved skin of hand eczema patients and healthy controls

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Accepted for publication 13 February 1991

Summary Basic physiological characteristics were examined in the uninvolved skin of 39 patients with hand eczema and in 39 healthy controls. Susceptibility to sodium lauryl sulphate (SLS)-induced irritant dermatitis was evaluated by the application of a single 24-h SLS patch test to the upper arm. Transepidermal water loss (TEWL) was measured by an evaporimeter, skin thickness by ultrasound A-scan, blood flow by laser-Doppler flowmetry and skin colour by a chroma meter using the L*a*b* system of the Commission Internationale de l'Éclairage (CIE). No difference in basal TEWL values was found between patients and controls. A decreased skin thickness was found in those with hand eczema as compared to the controls. The hand eczema patients had significantly increased L* and decreased b*-values compared to controls, indicating a more 'fair' skin. Susceptibility to SLS was increased only in patients with acute eczema, indicating that the presence of an active eczema increases the reactivity to irritants of distant uninvolved skin.

The susceptibility of the skin of patients with hand eczema to irritant chemicals was studied extensively by Bjørnberg[1] who found no increase in skin reactivity to primary irritants. Several factors have been suggested as predictors of sensitive skin. Atopic dermatitis has been proven to be of major importance in the development of irritant contact dermatitis,[2] but basic physiological characteristics such as an impaired barrier function of the stratum corneum,[3,4] fair skin[5] and the number of cell layers in the stratum corneum[6] have also been suggested as risk factors.

The aims of this study, using non-invasive measuring methods, were to assess the presence of some of these risk factors and to examine the response to an irritant stimulus in patients with hand eczema compared to matched controls.

Methods

Subjects

Thirty-nine patients with hand eczema (eight males and 31 females; median age 31 years, range 21–45 years) were included in the study. The patients were recruited from the out-patient dermatological clinic, Rigshospitalet, Copenhagen ($n=32$), and from a private skin clinic in Copenhagen ($n=7$). Patients with eczema in areas other than the hand or with another major skin disease were excluded from the study. Patients on systemic medications or having light therapy were also excluded, although those having treatment of the hands with topical steroids or tar were included. Duration of eczema was calculated from the first appearance of the disease, irrespective of intervening periods of healing. None had had the disease for less than 3 months, six patients had suffered from hand eczema from 3–6 months and 16 patients for more than 5 years. The patients were classified as having (i) current active eczema (presence of vesicles and erythema), (ii) current chronic eczema (presence of hyperkeratosis and desquamation, no vesicles and no marked erythema) or (iii) healed hand eczema (no eczema lesions for at least 3 months). Using the diagnostic criteria of Hanifin and Rajka,[7] patients were also classified according to whether or not they had a past history of atopic dermatitis. None of the patients in adulthood had manifestations of atopic dermatitis apart from hand eczema. Patients were patch tested using the standard series of the ICDRG (International Contact Dermatitis Research Group) and additional tests, and positive allergic reactions were found in 17 patients. One subject did not want to be patch-tested.

Thirty-nine age- and sex-matched healthy subjects with no history of atopic dermatitis or other skin diseases served as controls (median age 30 years, range 21–45 years). None had used a solarium. The study was carried

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out in the period December–April, and each patient and their matched control were examined within 4 weeks of each other. Females were not tested prior to or during the menstrual phase (from day 25 until day 5 in the menstrual period).[8]

Informed consent was obtained from all participants, and the study was approved by the local ethical committee.

Test procedure

The flexor side of the upper arm was chosen as the test region. Closed patch tests with 60 μl of aqueous solutions of 0·5% SLS (SLS Sigma, 99% purity) on filter discs were applied using large Finn chambers® on Scanpore® tape. Adjacent normal skin served as the control. The test chambers were removed after 24 h, and the test reactions evaluated 1 h later.

The reactions were evaluated by visual scoring according to the following scale: 0, no reaction; $\frac{1}{2}$, slight or very weak erythema; 1, weak erythema, possible slight infiltration; 2, marked erythema, infiltration, possible vesicles and crusting; 3, pronounced erythema, infiltration, possible vesicles, bullae, pustules and/or pronounced crusting.

The following non-invasive bioengineering methods were used to measure basic physiological parameters (prevalues) and to quantify the skin response to SLS (measurements performed 1 h after removal of patch tests).

Transepidermal water loss (TEWL)

TEWL was measured using the Evaporimeter (Servo Med® EP1, Stockholm, Sweden).[9] A boundary layer develops around the skin in which a water vapour gradient exists between the skin surface and ambient air. The sensors of the Evaporimeter (pairs of hygro-sensors and thermistors), mounted in the open chamber of the probe, determine the water vapour pressure gradient of this boundary layer in order to quantify the diffusion of water through the skin, i.e. the TEWL. The probe was hand-held using an insulating glove to avoid heating of the probe. Values were displayed on a pen-recorder and the mean value during the period 30–45 s after application of the probe on to the skin was read. A protection cover (no. 2107, supplied with the Evaporimeter) was used and all measurements performed inside an incubator to avoid convection of air, according to the guidelines of the Standardization Group of the European Society of Contact Dermatitis.[10]

Skin thickness

This was measured by a 20-MHz A-mode-pulsed-ultrasound scanner (Dermascan A®, Cortex Technology, Hadsund, Denmark).[11,12] By measuring the distance between the acoustic echoes from the stratum corneum and from the dermis/subcutis interface, respectively, the thickness of the epidermis and dermis together was measured. For calculation of the skin thickness an acoustic velocity of 1580 m/s was used.

Superficial blood flow

This was measured by a laser-Doppler flowmeter (Periflux Pf2B®, Perimed, Stockholm, Sweden), the operating principles for which were described by Tenland.[13] The instrument was adjusted to a bandwith of 20 Hz–12 kHz and again at 10 kHz. To keep the probe stable during measurements a 2·5 (w) × 10 (l) × 1·5 cm (h) plastic block with a hole for the probe and velcro straps for fastening around the arm was used. This kept the probe immovable during measurements, without the use of adhesive tape.

Skin colour

This was measured by a Minnolta Chroma Meter CR-200 (Osaka, Japan). The skin surface colour is quantified using the standard tristimulus system recommended by CIE (Commission Internationale de l'Éclairage).[14] The colour is expressed in a three-dimensional coordinate system. Luminance (L*) expresses the brightness (integrated reflection of light from the surface) ranging from total black (low values) to pure white (high values). a* and b* are the two colour coordinates: a* represents the colour range from green (−) to red (+), and b* the colour range from blue (−) to yellow (+). The saturation of the colour L*a*b* system is calculated as Chroma $C=\sqrt{a^{*2}+b^{*2}}$, and the difference in total colour is calculated as $\Delta E=\sqrt{\Delta a^{*2}+\Delta b^{*2}+\Delta L^{*2}}$. Changes in a*-values were found to be useful for the assessment of patch-test reactions[15-17] and for the response of skin to UVB.[18] Measurement of the integrated reflection of light has been used successfully to quantify blanching,[19] and for the assessment of constitutional skin colour.[20] The purpose of the Minolta Colorimeter is to register colour as it is perceived by the human eye, and the true colour of the skin is an admixture of the a*, b* and L* values.

All visual scorings and instrumental recordings were performed by the author. Disturbances in the laboratory were kept to a minimum during measurements. The relative humidity varied between 30 and 45%, and the room temperature was kept at 20–22°C. The skin temperature during measurements was in all instances within the range 29–31°C, measured on skin in the test region. Skin temperature measurements were performed using a skin thermometer with a hand held thermocouple probe, Comarck 2001® (Comarck Electronics, Rustington, U.K.).

Measurements were expressed as the mean value of two recordings (evaporimetry and colorimetry) or three recordings (laser-Doppler flowmetry and ultrasound). The variation found during quantification of irritant patch-test reactions by non-invasive methods and the comparisons between methods have previously been reported.[17,21]

Statistics

Wilcoxon's test for unpaired samples was used for comparison between groups. Two-way as well as one-way analysis of variance was used to examine the variation within the hand-eczema group; for analysis of 'visual scoring', data were rank transformed. Spearman's test was used for correlation studies.

Results

The median values for basic physiological characteristics for eczema patients and for controls are given in Table 1. Significantly thinner skin was found in patients with hand eczema than in their matched controls. With respect to skin colour as measured by the chroma meter, significantly increased L^*-values and significantly decreased b^*-values were found in hand eczema patients as compared to controls. No significant difference in chroma (C) was found between the groups ($P>0\cdot05$, data not shown). No significant differences between the two groups were found for basal TEWL or basal blood flow ($P>0\cdot05$).

The results of exposure of the skin to SLS in patients with hand eczema and controls are shown in Table 2. The response was significantly more pronounced in patients with hand eczema than controls as evaluated by visual scoring, measurement of TEWL, and measurement of the increase in skin thickness, while measurement of blood flow showed no significant differences between the two groups. No significant difference in change of skin colour was found between patients and controls, either when comparing light reflection or colour coordinates ($L^*a^*b^*$, Table 2), or when comparing the chroma (C) or total colour difference (ΔE) for the two groups, respectively ($P>0\cdot05$, data not shown). The median values of visual scoring, TEWL, increase in skin thickness, blood flow and skin colour after exposure to SLS, in hand eczema patients grouped according to current state of eczema are shown in Table 3, as are the numbers of patients with presence/absence of a past history of atopic dermatitis. By two-way analysis of variance it was found that classification according to atopy had no significant effect for either basic physiological parameters or for values obtained after exposure to SLS ($P>0\cdot05$), whereas there was a statistically significant variation according to the current state of eczema for values obtained after exposure to SLS ($P<0\cdot05$). Because no variation related to a past history of atopic dermatitis was found, variation related to the current state of eczema was examined by a one-way analysis of

Table 1. Basic physiological characteristics of eczema patients and controls

	Hand eczema ($n=39$)	Controls ($n=39$)	P
Basal TEWL (g/m^2h)	5·4 (4·2–6·1)†	5 0 (4·5–5·6)	NS
Basal skin thickness (mm)	0·92 (0·86–0·97)	0·97 (0·92–1·02)	<0 005
Basal blood flow (a.u.)	7 (5–9)	6 (5–8)	NS
Skin colour			
L^*	66·3 (64·5–67·8)	64·3 (61·8–66·1)	<0·01
a^*	5·2 (4·0–6·6)	5·7 (5·2–7·4)	NS
b^*	12·8 (11·1–14·4)	14·4 (12·6–16·4)	<0·05

† Median values and 25/75 percentiles for the basic physiological parameters. P-values for differences between patients and controls are calculated by Wilcoxon's test for unpaired samples. NS indicates $P>0\cdot05$.

Table 2. Skin response to SLS

	Hand eczema ($n=39$)	Controls ($n=39$)	P
Visual scoring	1 ($\frac{1}{2}$–2)†	1 ($\frac{1}{2}$–1)	<0·05
Δ TEWL (g/m^2h)	12·3 (8·5–16·5)	8·8 (7·1–12·5)	<0·05
Δ Skin thickness (mm)	0·30 (0·19–0·41)	0·22 (0·16–0·26)	<0·05
Δ Blood flow (a.u.)	15 (5–29)	7 (3–25)	NS
Skin colour			
Δ L*	−1·4 (−2·9–−0·1)	−1·5 (−2·6–−0·33)	NS
Δ a*	2·7 (1·5–5·3)	3·3 (0·8–4·4)	NS
Δ b*	0·4 (−0·5–0·87)	0·3 (−0·6–1·16)	NS

† Median values and 25/75 percentiles for values obtained after exposure to SLS. Δ indicates delta values calculated as value obtained from SLS-exposed skin − basal value. *P*-values for differences between patients and controls are calculated by Wilcoxon's test for unpaired samples. NS indicates $P>0{\cdot}05$.

Table 3. Skin reactivity to SLS in patients with acute, chronic and healed hand eczema

	Acute ($n=16$)	Chronic ($n=14$)	Healed ($n=9$)	P
Atopy/non atopy (n)	4/12	5/9	1/8	
Visual scoring	2** (2–3)†	$\frac{1}{2}$ ($\frac{1}{2}$–1)	1 ($\frac{1}{2}$–1)	<0·01
SLS-TEWL (g/m^2h)	23·0** (17·2–29·7)	14·2 (11·9–17·7)	15·5 (11·5–21·2)	<0·01
Δ Skin thickness (mm)	0·51* (0·34–0·68)	0·22 (0·15–0·27)	0·25 (0·19–0·33)	<0·01
Blood flow (a.u.)	38** (15–110)	17 (9–24)	13 (9–20)	<0·01
Skin colour				
L*	63·3 (62·2–65·4)	64·6 (61·5–66·9)	63·6 (62·7–66·4)	NS
a*	10·3* (9·3–11·8)	7·3 (4·9–9·7)	6·3 (6·2–7·2)	<0·01
b*	12·2 (10·7–14·4)	12·8 (10·0–15·0)	14·6 (12·7–16·9)	NS

† Median values and 25/75 percentiles for values obtained after exposure to SLS. The *P*-value indicates the presence of a variation between the groups when examined by a one-way analysis of variance. The number of patients with presence/absence of a past history of atopic dermatitis is given in the first line. * Statistically significant when compared to normal controls (* = $P<0{\cdot}05$, ** = $P<0{\cdot}005$).

variance (Table 3). Median values were higher in the group with acute eczema than in the group with chronic/healed eczema. Comparing patients with acute eczema ($n=16$) to controls, a significant difference was found in skin susceptibility to SLS as assessed by visual scoring, measurement of TEWL, increase in skin thickness and blood flow ($P<0{\cdot}005$). With respect to skin colour, patients with acute eczema had increased a* colour coordinate values as compared to controls ($P<0{\cdot}05$), while no difference in b*- or L*-values was found ($P>0{\cdot}05$). No significant difference in chroma (C) was found between patients with acute eczema and controls ($P>0{\cdot}05$, data not shown). A comparison of patients with chronic ($n=14$) or healed ($n=9$) eczema with controls showed no difference in skin susceptibility to SLS ($P>0{\cdot}05$).

The correlation between basal TEWL and TEWL after exposure to SLS for patients with eczema as well as controls is shown in Figure 1 ($R=0{\cdot}79$ and 0·73, respectively, $P<0{\cdot}0005$). The correlation between basal TEWL and Δ TEWL was $R=0{\cdot}73$ and 0·61 for patients and controls, respectively.

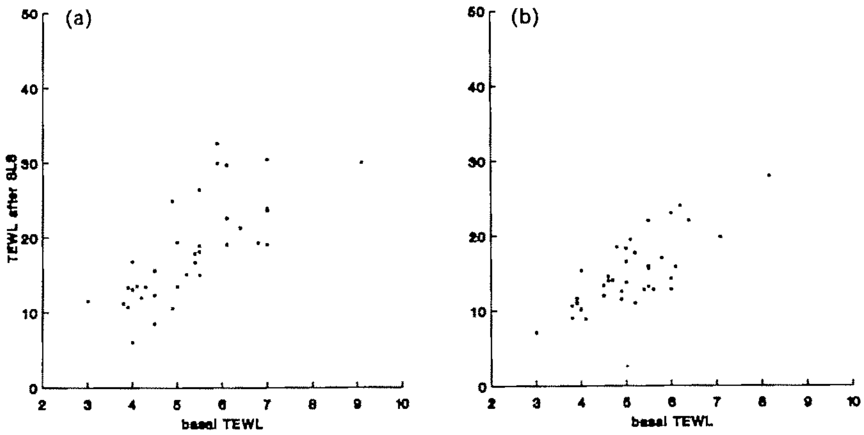

Figure 1. Correlation between basal TEWL (g/m^2 h)and TEWL after exposure to SLS (g/m^2 h) for patients with (a) hand eczema and (b) controls. The Spearman coefficient of correlation was $R=0\ 79$ ($P<0{\cdot}0005$) and $R=0{\cdot}73$ ($P<0{\cdot}0005$), respectively.

No significant differences were found between patients with and without relevant reactions to allergic patch tests, for any of the measured parameters ($P>0{\cdot}05$).

Discussion

Basal TEWL was not significantly increased in patients with hand eczema as compared to controls. This observation together with an earlier report on patients with irritant contact dermatitis[22] demonstrates that a generalized defect in the skin barrier is not present in patients with hand eczema. Basal TEWL as a predictor of skin susceptibility to irritants has been previously reported,[3,4] although this remains controversial.[23,24] A positive correlation between basal TEWL and SLS-TEWL as well as basal TEWL and Δ TEWL was confirmed in this study. However, basal TEWL values obtained from the arm do not necessarily reflect the susceptibility of the skin of the hands.

Although higher basal TEWL values for patients with atopic dermatitis than for non-atopics have been reported,[22,25] in this study no variation in basal TEWL related to presence/absence of a past history of atopic dermatitis was found. Also, as expected from the basal TEWL values, no increased responsiveness to SLS among patients with a past history of atopic dermatitis was found. Patients included in this study with a past history of atopic dermatitis had had no manifestation of the disease since childhood, apart from hand eczema, which may explain the difference between the present results and earlier reports. The normal level of basal TEWL found in these patients brings into question the concept of a constitutional defect of the barrier function in patients with atopic dermatitis, and indicates an association between basal TEWL and the (sub)clinical activity of the disease.

Increased basal TEWL measurements in the uninvolved skin of patients with acute dermatitis have been reported.[26] This was not confirmed statistically in the present study, although there was a trend toward higher values for basal TEWL in patients with acute eczema as compared to controls ($P=0{\cdot}06$).

The basal skin thickness was found to be significantly less in patients with hand eczema than in their matched controls, with no significant correlation with a history of atopy or the current state of eczema. This new finding may possibly reflect a generalized structural anomaly in the skin of patients with hand eczema. The thickness of the stratum corneum was earlier found to be of importance in the pathophysiology of sensitive skin.[6] When skin thickness is quantified by ultrasound A-scan the measurement includes the epidermis together with the dermis. The stratum corneum thus represents only a small fraction of the total measurement and a small variation in the numbers of stratum corneum cell layers is beyond the detection limit of the method. Treatment with topical steroids, known to induce dermal thinning,[27] was allowed in this study in patients with acute and chronic eczema. All patients were or had been using topical steroids, but on the hands only. It is theoretically possible that treatment with topical steroids on the hands will influence skin thickness on the upper arm, and explain the decreased skin thickness in the group with hand eczema, but further studies are necessary.

Fair-skinned subjects were reported to be more susceptible to irritants than dark-complexioned subjects, and the determination of the minimum erythema dose (MED) was found to be useful for prediction of susceptibility to irritants.[5] In the present study the hand eczema group showed increased brightness of the skin surface (L*) and decreased b* colour coordinates as measured on normal skin, compared to controls. This indicates that the group was more 'fair-skinned' than the control group, because it has been demonstrated that tanning of the skin leads to a decrease in L*-values and increase in b*-values.[18] b*-values were found to be even more suitable than L*-values for assessment of tanning, but this conclusion may have resulted from the presence of erythema leading to changes in the L*-values.[18] Recently the integrated reflection of light from the skin surface was found to be a good predictor for the dose-response measurements of UV-induced erythema and pigmentation.[20] However, the light reflection from the skin surface (L*) is a complex factor influenced by chromophores in the skin (melanin, haemoglobin, bilirubin, carotene), but also by factors which change the skin surface reflectiveness such as skin dryness or roughness and possibly sebum excretion. Also, structural differences in the epidermis and dermis will influence the penetration, scattering and reflection of the light, and thus influence the L*-value.[28] A more accurate determination of skin colour could perhaps be obtained using an instrument based on the principles of Farr and Diffey.[29]

The hand eczema group was found to be more susceptible to SLS than their matched controls (Table 2). The analysis of variance showed that the increased skin susceptibility of uninvolved skin to SLS varied significantly within the hand eczema group, being consistently higher in those patients with acute eczema (Table 3). This agrees with previous reports that the presence of even a localized eczema may cause an increased susceptibility to at least some irritants.[1] When comparing skin reactivity to SLS in patients with chronic or healed eczema with controls, no enhanced reactivity was found, and a generalized increase in the susceptibility of the skin to SLS in patients with hand eczema could not be demonstrated. Because no significant variation in basal TEWL was found, the increased reactivity to SLS in patients with acute eczema is more likely to be due to a generally enhanced inflammatory reactivity of the skin than to an impaired barrier function.

Acknowledgment

The author is indebted to Drs P.E.Osmundsen, and B.Aastrup, Skin Clinic, Brønshøj, Copenhagen, for referring patients for the study, and to Dr Aage Vølund, the Danish Medical Research Council, for helping with the statistical calculations.

References

1 Bjørnberg A. *Skin Reactions to Primary Irritants in Patients with Hand Eczema*. Göteborg: Isacson, 1968.

2 Meding B, Swanbeck G. Epidemiology of different types of hand eczema in an industrial city. *Acta Derm Venereol (Stockh)* 1989; **69**: 227–33.

3 Murahata RI, Crowe DM, Roheim JR. The use of transepidermal water loss to measure and predict the irritation response to surfactants. *Int J Cosmet Science* 1986; **8**: 225–31.

4 Pinnagoda J, Tupker RA, Coenraads PJ, Nater JP. Prediction of susceptibility to an irritant response by transepidermal water loss. *Contact Dermatitis* 1989, **20**: 341–6.

5 Frosch PJ, Wissing C. Cutaneous sensitivity to ultraviolet light and chemical irritants. *Arch Dermatol Res* 1982; **272**: 269–78.

6 Frosch PJ, Kligman AM. Rapid blister formation in human skin with ammonium hydroxide. *Br J Dermatol* 1977; **96**: 461–73.

7 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; Suppl **92**: 44–7.

8 Agner T, Damm P, Skouby SO. Menstrual cycle and sensitive skin. *J Am Acad Dermatol* 1991; **24**: 566–70.

9 Nilsson GE. On the measurement of evaporative water loss. *Medical Dissertations* No. 48. *Linköping University*, 1977.

10 Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis* 1990; **22**: 164–78.

11 Serup J. Localized scleroderma (Morphoea). Thickness of the sclerotic plaques as measured by 15 MHz pulsed ultrasound. *Acta Derm Venereol (Stockh)* 1984; **64**: 214–19.

12 Serup J. *Ten Years Experience with High Frequency Ultrasound Examination of the Skin: Development and Refinement of Technique and Equipments*. (Altmeyer P, ed). Bochum: Karger, (in press)

13 Tenland T. On laser Doppler flowmetry. Methods and microvascular applications. *Linköping Medical Dissertations* No. 136. Linköping University, 1982.

14 Robertson AR. The CIE 1976 color difference formulas. *Color Research and Application* 1977; **2**: 7–11.

15 Serup J, Agner T. Colorimetric quantification of erythema. *Clin Exp Dermatol* 1990; **15**: 267–72.

16 Babulak SW, Rhein LD, Scala DD *et al.* Quantification of erythema in a soap chamber test using the Minnolta Chroma (reflectance) Meter: comparison of instrumental results with visual assessments. *J Soc Cosmet Chem* 1986; **37**: 475–9.

17 Agner T, Serup J. Sodium lauryl sulphate for irritant patch testing: A dose response study using bioengineering methods for determination of skin irritation. *J Invest Dermatol* 1990; **95**: 543–7.

18 Seitz JC, Whitmore CG. Measurement of erythema and tanning responses in human skin using a tri-stimulus colorimeter. *Dermatologica* 1988; **177**: 70–5.

19 Queille-Roussel C, Poncet M, Schaefer H. Quantification of skin colour changes induced by topical corticosteroids on normal skin using the Minolta chroma meter. Abstract presented at the 8th International Symposium on Bioengineering and the Skin, Stresa, Italy, June 1990.

20 Westerhof W, Estevez-Uscanga O, Meens J *et al.* The relation between constitutional skin colour and photosensitivity estimated from UV-induced erythema and pigmentation dose-response curves. *J Invest Dermatol* 1990; **94**: 812–16.

21 Agner T, Serup J. Individual and instrumental variation in patch test reactions. *Clin Exp Dermatol* 1990; **15**: 29–33.

22 Van der Valk PGM, Nater JP, Bleumink E. Vulnerability of the skin to surfactants in different groups of eczema patients and controls as measured by water vapour loss. *Clin Exp Dermatol* 1985; **10**: 98–103.

23 Freeman S, Maibach HI. Study of irritant contact dermatitis produced by repeat patch testing with sodium lauryl sulphate and

assessed by visual methods, transepidermal water loss and laser Doppler velocimetry. *J Am Acad Dermatol* 1988; **19**: 496–502.

24 Wilhelm KP, Maibach HI. Susceptibility to irritant dermatitis induced by sodium lauryl sulphate. *J Am Acad Dermatol* 1990; **23**: 122–4.

25 Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. *Acta Derm Venereol (Stockh)* 1985; **65**: 102–5.

26 Shahidullah M, Raffle EJ, Rimmer AR, Frain-Bell W. Transepidermal water loss in patients with dermatitis. *Br J Dermatol* 1969; **81**: 722–30.

27 Tan YC, Marks R, Payne P. Comparison of xeroradiographic and ultrasound detection of corticosteroid induced dermal thinning. *J Invest Dermatol* 1981; **76**: 126–8.

28 Rox Anderson R, Parrish JA. The optics of human skin. *J Invest Dermatol* 1981; **77**: 13–19.

29 Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation *Br J Dermatol* 1984; **111**: 673–82.

British Journal of Dermatology (1991) **125**, 147–154.

ADONIS 000709639100185 2

The differentiation and proliferation of newly formed epidermis on wounds treated with cultured epithelial allografts

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Accepted for publication 13 February 1991

Summary Fifteen patients, eight with burn or scald wounds and seven with split-thickness donor sites, were treated with cultured epithelial allografts. Skin was obtained from HIV-negative donors undergoing circumcision and sheets of epithelium were cultured using the 3T3 feeder method. Multiple post-operative biopsies were performed at various time intervals and stained with a panel of monoclonal antibodies against cytokeratins, involucrin, transferrin receptor and epidermal growth factor receptor. Fresh cultured epithelial sheets, normal skin, standard treated donor sites and burns treated with autografts were also studied. Cytokeratin-10 expression was not observed at treated sites until 4 weeks post-grafting, when normal suprabasal levels were observed. Cytokeratins 13 and 16, usually observed in highly proliferative states such as psoriasis, were observed in epithelial-treated sites for up to 6 months. Other proliferation markers such as Ki67 and transferrin receptor were only expressed 2–3 weeks post-operatively. Involucrin, a marker of keratinocyte terminal differentiation, was expressed throughout newly formed epidermis until 15 weeks, when the normal pattern of granular expression was observed. These results indicate that although the cultured 'allograft' does not survive, it may modulate the proliferation and differentiation of spontaneously regenerating epithelium.

Cultured epithelial allografts (keratinocyte allografts) have been used in the successful treatment of burns and other wounds such as ulcers in a number of centres.[1–3] It has recently been shown that the allograft does not survive, but is replaced by host epithelial cells possibly derived from dermal remnants.[4,5] Although the keratinocyte allograft may promote more rapid wound healing,[5] little is known about the antigenic characterization of the newly epithelialized area.

Cytokeratins are a complex group of 20 polypeptides that form intermediate filaments in human epithelial cells.[6] The cytokeratin profile of keratinocytes is closely related to differentiation and may be used to evaluate the clinical origin and state of the cell. Cytokeratins 1 and 10, that are characteristic of keratinizing tissues, are observed in the suprabasal layers while the proliferating basal keratinocytes express cytokeratins 5 and 14.[7] The cytokeratin pattern may also be modulated by environmental conditions and disease.[8] The aim of this study was to examine the effect of the keratinocyte allograft on the newly formed epidermis using a panel of monoclonal antibodies against cytokeratins and other proliferation and differentiation markers.

Methods

Patients

Six adults (age range 19–68 years) and two children (aged 2 and 7 years) were treated for partial thickness burn injuries and seven patients (age range 36–78 years) for donor site defects, at a site where a superficial split-skin graft had been taken. Split skin meshed autografts were applied as well as cultured epithelium to burn injuries.

Keratinocyte culture

Allogeneic keratinocytes were grown from paediatric foreskins from HIV-negative donors, using a modification of previously published methods.[9,10] Briefly, after

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Table 1. Details of biopsies

Wound + treatment	Number of Patients	Day of post-transplantation biopsy (number taken)			
Adult partial thickness burn—epithelial allograft	6	14 (2)	26 (3)	28 (2)	
		49 (3)	65 (1)	73 (2)	
		100 (2)	180 (1)	360 (1)	
Child partial thickness burn—epithelial allograft	2	8 (1)	14 (1)	28 (1)	40 (1)
Adult partial thickness burn—marked split-skin graft	2	14 (1)	28 (1)	65 (1)	73 (1)
		100 (1)	180 (1)	360 (1)	
		14 (2)	28 (2)	44 (2)	50 (3)
Donor site—epithelial allograft	7	90 (2)	180 (2)	360 (2)	
Donor site—standard treatment	7	14 (2)	28 (2)	44 (2)	50 (3)
		90 (2)	180 (2)	360 (2)	

overnight incubation in 0·25% dispase (Boehringer, Mannheim, Germany) the epidermis was peeled from the dermis and trypinized at 37°C to obtain a single cell suspension, which was plated on to irradiated 3T3 fibroblasts. Keratinocyte sheets for grafting were taken at the second passage.

Surgical application and treatment

Partial thickness burns were treated by the dermabrasion method of Holmes and Rayner.[11] The burn was treated by exposure or dressings until the fourteenth day post-injury, when areas of deep dermal burns were lightly dermabraded until punctate bleeding occurred. The epithelial sheet was placed basal side down to cover the dermabraded areas and secured in place with staples. The wound was covered with petroleum gauze, cotton wool and crepe bandages, which were changed after 10 days and thereafter every 5 days. At donor split-skin autograft sites, epithelial sheets were placed directly on to the sites and secured as above. Control areas were treated with petroleum gauze and dressings alone which were changed after 1 week. Punch biopsies (4 mm) were taken from the centre of allografted and control sites such as meshed split-thickness skin graft sites covered with meshed autograft, standard-treated donor sites and healthy skin at various time intervals (Table 1). Early biopsies up to 14 days post-grafting were taken from healing areas, whereas all later biopsies were taken from healed areas.

Immunohistochemistry

Biopsies were snap frozen and 5–7 μm cryostat sections were mounted on to gelatin-coated slides, acetone fixed for 20 min and stained by the unlabelled peroxidase antiperoxidase (PAP) method. The sources, specificities and dilutions of the primary layer monoclonal antibodies (MAb) are shown in Table 2. The MAbs were applied to air-dried sections for 60 min at room temperature. Controls included the replacement of primary antibody with TBS or normal mouse immunoglobulin. After washing these were incubated with rabbit anti-mouse Ig (Dakopatts 1:20) in 20% normal human AB serum for 30 min, followed by a 30-min incubation with 1/100 dilution of PAP complex (Dakopatts). The bound peroxidase was visualized using the diaminobenzidine/H_2O_2 reaction. Sections were counterstained in Harris haematoxylin, dehydrated and mounted in DPX. The intensity of staining was assessed and graded on a scale from 0 to + +. The control slides were uniformly negative.

Results

Clinical assessment

In this study all epithelial sheets were placed on a clean dermal-bed surface that had a regular honeycomb appearance of a white background with small red dots. When first observed at 10 days there were no signs of rejection (erythema or blistering) and the surface appeared smooth, translucent and moist, with the allograft being sloughed off. At this stage epithelialization was observed to occur from the edges and epithelial islands within the site; full epithelialization occurred between 14 and 21 days. A study using ABO typing and sex-mismatch Y probe techniques showed that healed areas consisted of donor keratinocytes alone (W.Kaawash, personal observation) which agrees with

Table 2. Monoclonal antibody panel

Antibody	Isotype	Specificity	Source
RPN 1160	IgG2b	Cytokeratin 18	Amersham
RPN 1162	IgG1	Cytokeratin 7	Amersham
RPN 1164	IgG1	Type II cytokeratins	Amersham
RPN 1165	IgG2b	Cytokeratin 19	Amersham
RPN 1166	IgG1	Cytokeratin 8	Amersham
K 8.12	IgG1	Cytokeratin 13, 16	Sigma
K 8.13	IgG2a	Cytokeratin Range	Sigma
K 8.60	IgG1	Cytokeratin 10, 11	Sigma
Ks 13.1	IgG1	Cytokeratin 13	Cymbus Bioscience
CAM 5.2	IgG2a	Cytokeratin 8, 18, 19	Becton Dickinson
34 BE12	IgG1	Cytokeratin 5, 10, 11	Dr A.Gown*
GB5	IgG1	Strata granulosum spinosum + germinatum	Seralab
GB6	IgM	Basal keratinocytes	Seralab
Ber T9	IgG1	Transferrin receptor	Dakopatts
Ki67	IgG1	Proliferating cell	Dakopatts
EGF-r	IgG2b	Epidermal growth factor receptor	Amersham
Vim 13.2	IgM	Vimentin	Serotec
Involucrin (polyclonal)	IgM	Involucrin	Dr F.Watt†

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previous reports.[4,5] Donor sites that had been treated with epithelial sheets and dressings alone were compared at 1 week. The site that had received an allograft was epithelialized to a greater extent than the standard treated site, sometimes as much as 60–90%. Hypertophic scarring, pain and itching were all reduced at the allograft sites.

Cytokeratin expression

The cytokeratin expression observed in newly epithelialized skin after treatment with keratinocyte allografts is shown in Table 3. As the allograft does not 'take' in the immunological sense but appears to act as a biological dressing[4,5] all observations were made on keratinocytes of host origin. The antibody RPN1164 (against type II cytokeratins) that is negative in adult skin, was strongly positive in allograft treated sites up to 6–7 weeks, with staining becoming paler by 10 weeks and assuming the normal pattern by 12 weeks. However, autograft-treated sites and control sites were negative with this antibody.

An interesting staining pattern was observed with the antibody 34BE12 against cytokeratins 5, 10 and 11. In

Table 3. Cytokeratin distribution at wound sites

Cytokeratin	Distribution	Normal skin	Epidermal sheet	Keratinocyte allograft* sites 1–8	Keratinocyte allograft* sites 8→	Autograft sites	Untreated donor site
5	Basal cell	+	+	++	++	+	+
7	Suprabasal	–	+	–	+/–	–	–
10,11	Suprabasal	+	+	–	+	+	+
13	Suprabasal	–	+	++	++	+	++
13,16	Suprabasal	–	+	++	++	+	+
19		–	+	–	–	–	–
Type II	Suprabasal	–	–	+	++	+	+

–, no staining; +, low-intensity staining; ++, high-intensity staining. * At number of weeks post-grafting

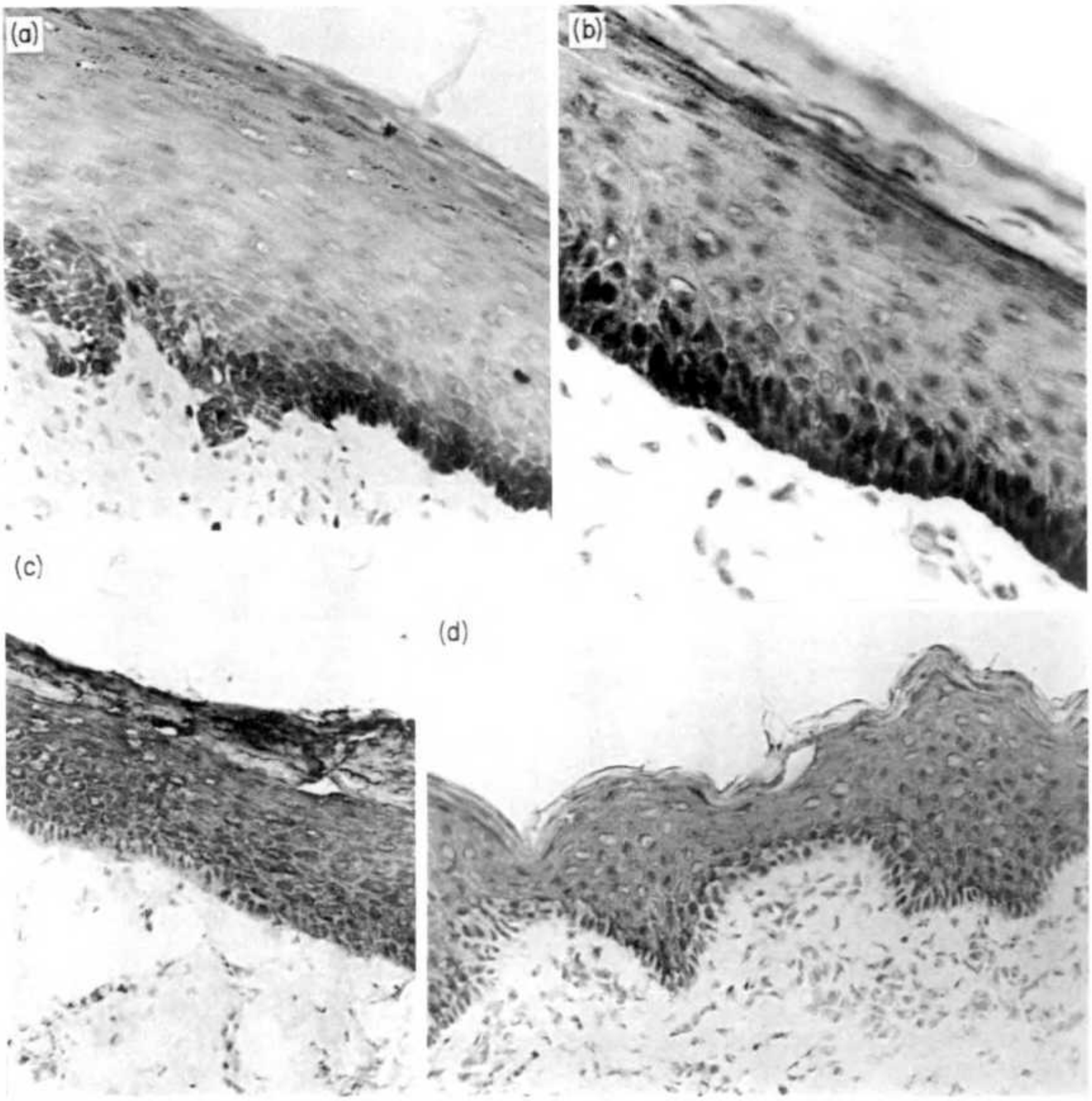

Figure 1. Staining pattern observed with the monoclonal antibody 34BE12 against cytokeratins 5, 10 and 11 (a) on keratinocyte allograft-treated burn 4 weeks post-grafting, showing positive basal cells and negative suprabasal cells (×400); (b) on keratinocyte allograft-treated burn 10 weeks post-grafting, showing a dark basal cell staining and lighter suprabasal staining pattern (×400); (c) untreated donor site 9 days post-grafting showing positive cells distributed throughout the epidermis (×200); and (d) normal skin, showing positive cells distributed throughout the epidermis (×200).

normal and standard-treated donor site skin, staining throughout the epidermis was observed, but in the allograft sites only basal cells showed positive staining up to 10 weeks, after which the normal pattern was observed (Fig. 1). Conversely, K8.60 against cytokeratins 10 and 11 did not stain newly formed epidermis until 10 weeks post-grafting, or 7 weeks post-epithelialization. As cytokeratin 5 is usually present on basal keratinocytes[7] these results would indicate that cytokeratin 5 is expressed early in wound healing, while cytokeratin 10 appears later.

Cytokeratins 13 and 16 were also strongly expressed in healing epidermis, but this was observed at all sites regardless of treatment. However, persistance of these markers varied. In allograft-treated burn wounds these cytokeratins were still observed at 6 months post-grafting, i.e. over 5 months since epithelialization, whereas in allograft-treated donor sites expression was weak or negative by 3 months and in control sites by 28 days. Although cytokeratin 19 was expressed in the fresh epidermal sheet, all biopsy material was negative for this cytokeratin. All samples were negative for

Table 4. Expression of differentiation and proliferation markers at wound sites

Antibody specificity	Distribution	Normal skin	Epidermal sheet	Keratinocyte allograft* sites		Autograft site	Untreated donor site
				1–8	8→		
Ki67	Basal	–	+	++	+	+	+
	Suprabasal	–	–	+	–	–	–
Vimentin	Suprabasal	–	+	–	–	–	–
Transferrin receptor	Suprabasal	–	+	++	–	–	+
EGFr	Basal	+	+	++	+	+	+
	Suprabasal	++	+	++	++	++	++
Involucrin	Lower spinous	–	+	+	+/–	–	–
	Upper spinous	–	+	+	+	+	+
	Granular layer	++	–	+	++	+	+

–, no staining; +, low intensity staining, ++, high intensity staining. * At number of weeks post-grafting.

cytokeratins 7, 8 and 18, except a small number of early allograft sites (1–3 weeks) which were faintly positive for cytokeratin 7.

There were no differences observed between samples from young and old patients.

Proliferation and differentiation markers

Biopsies from the allografted sites were also studied for the expression of various antigens also involved in cell differentiation and proliferation (Table 4). Transferrin receptor and Ki67, both markers for proliferation (and not present in normal skin) were observed at allografted and untreated donor sites (Fig. 2). Transferrin receptor, which indicates the cell's requirements for iron, disappeared by 10 weeks at allografted sites and by 4 weeks at control sites, while Ki67, which reacts with proliferating cells, was still faintly visible at 6 months at treated sites and up to 3 months at untreated sites. Epidermal growth-factor receptor was strongly expressed in all samples, but no differences were detected between normal, treated and untreated skin with this technique.

Involucrin, a marker of keratinocyte terminal differentiation is present in the granular layer of normal skin. However, at early burn allograft sites (1–8 weeks) it was expressed throughout the epidermis (Fig. 3). By 10 weeks only the upper spinous and granular layer were positive and the normal pattern of distribution was observed by 15–16 weeks. In contrast, allograft-treated donor sites expressed involucrin in the upper spinous and granular layer for up to 8 weeks post-grafting, when normal distribution was observed. At meshed-skin autograft and standard-treated donor sites the normal pattern of involucrin distribution was observed.

Discussion

This study has demonstrated that at epithelial allograft-treated sites, the newly formed epidermis remains in a highly proliferative and undifferentiated state for 3–6 months post-grafting, despite a relatively normal clinical and histological appearance.[12,13] Previous studies have indicated that cultured keratinocyte allografts may promote wound healing by secreting cytokines, for example TGFα, a potent autocrine growth factor.[14–16] This cytokine has been shown to occur in high concentrations in the stratum corneum and is released promptly into an injury site via the corneal lateral diffusion pathway to prime wound healing.[17] However, the persistence of proliferative markers such as cytokeratin 13 and 16, usually observed in hyperproliferative diseases such as psoriasis,[8,18] long after the disappearance of the donor keratinocytes would indicate an initial effect perpetuated by the newly forming epidermis. This agrees with other studies which showed that cytokeratin 16 was present in regenerating skin,[19] persisted for up to 16 weeks at allografted ulcer sites[20–22] and up to 6 months at allografted deep dermal burns.[23] Although these cytokeratins are expressed during normal wound healing their disappearance usually coincides with epithelialization after 10–14 days.[20] The expression of cytokeratins 13 and 16 for up to 5 months post-

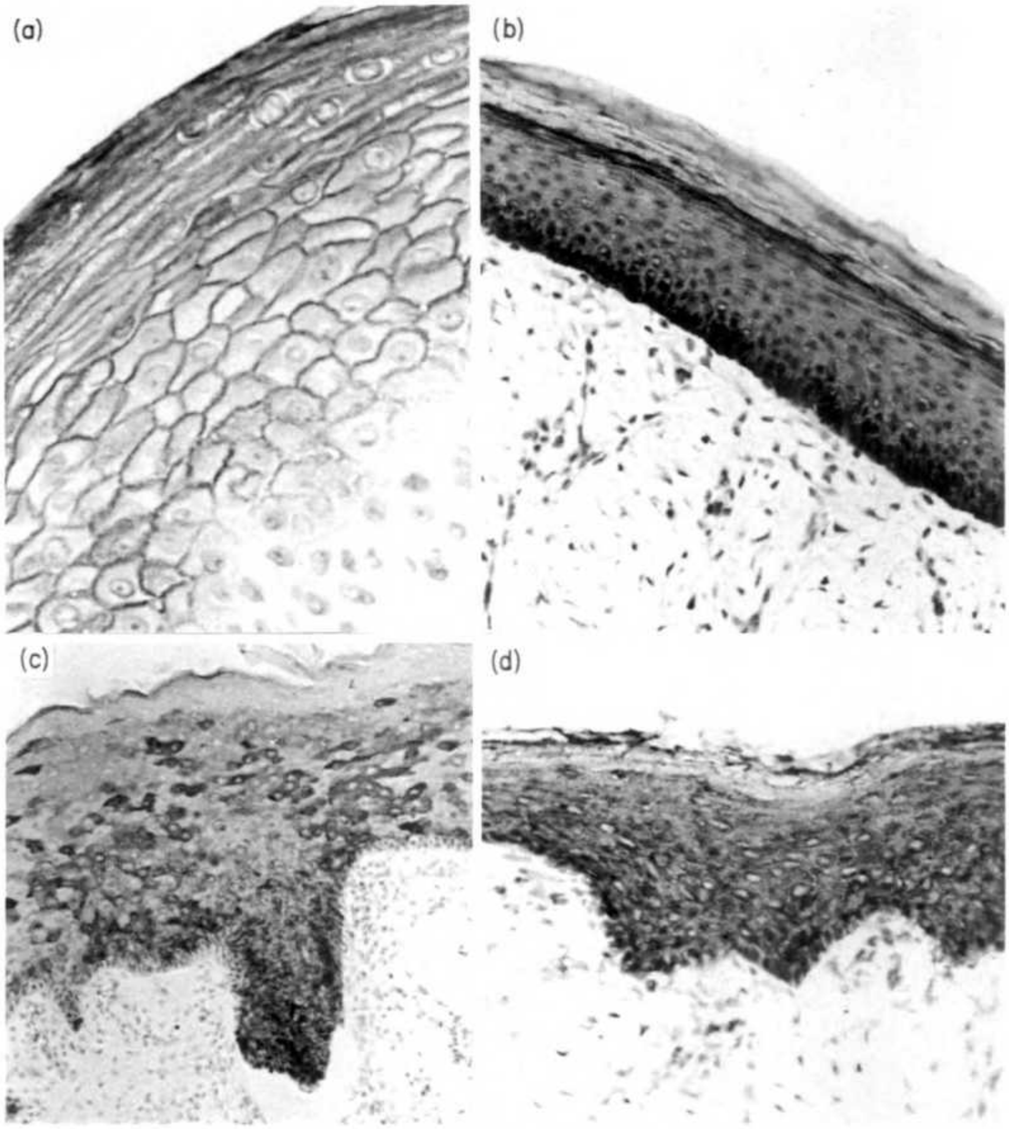

Figure 2. Staining pattern observed on keratinocyte allograft-treated sites (1–4 weeks post-grafting) with monoclonal antibodies against (a) transferrin receptor, showing positive upper spinous layer cells (× 400); (b) Ki67 (proliferating cell), showing dark basal cells and light supra basal cells (× 200); (c) cytokeratin 13, showing dark stained cells scattered throughout the epidermis (× 200); and (d) type II cytokeratins throughout the epidermis (× 200).

epithelialization, at allograft-treated burn sites and to a lesser extent at donor sites, may reflect prolonged wound healing that depends on the depth of the wound. The persistent expression of transferrin receptor and Ki67, that we observed, may lend further support to this hypothesis. The expression on treated sites of type II cytokeratins recognized by the antibody RPN1164 is of particular interest as this antibody is negative on normal adult skin but present early in foetal skin development (up to 17 weeks estimated gestational age).[24] A similar pattern was also observed with cytokeratin 7 that is also expressed during foetal development and in keratinocytes in culture. Therefore the presence of these markers on newly developing epidermis may indicate that cells go through a foetal stage when they first migrate to the wound site in response to cytokine release. These markers were absent in autograft and untreated sites, indicating that the keratinocyte allograft may be involved in the expression of these markers.

The presence of cytokeratin 5, but not 10, in the early keratinocyte-treated biopsy material, but not the controls, points to a highly proliferative but undifferentiated

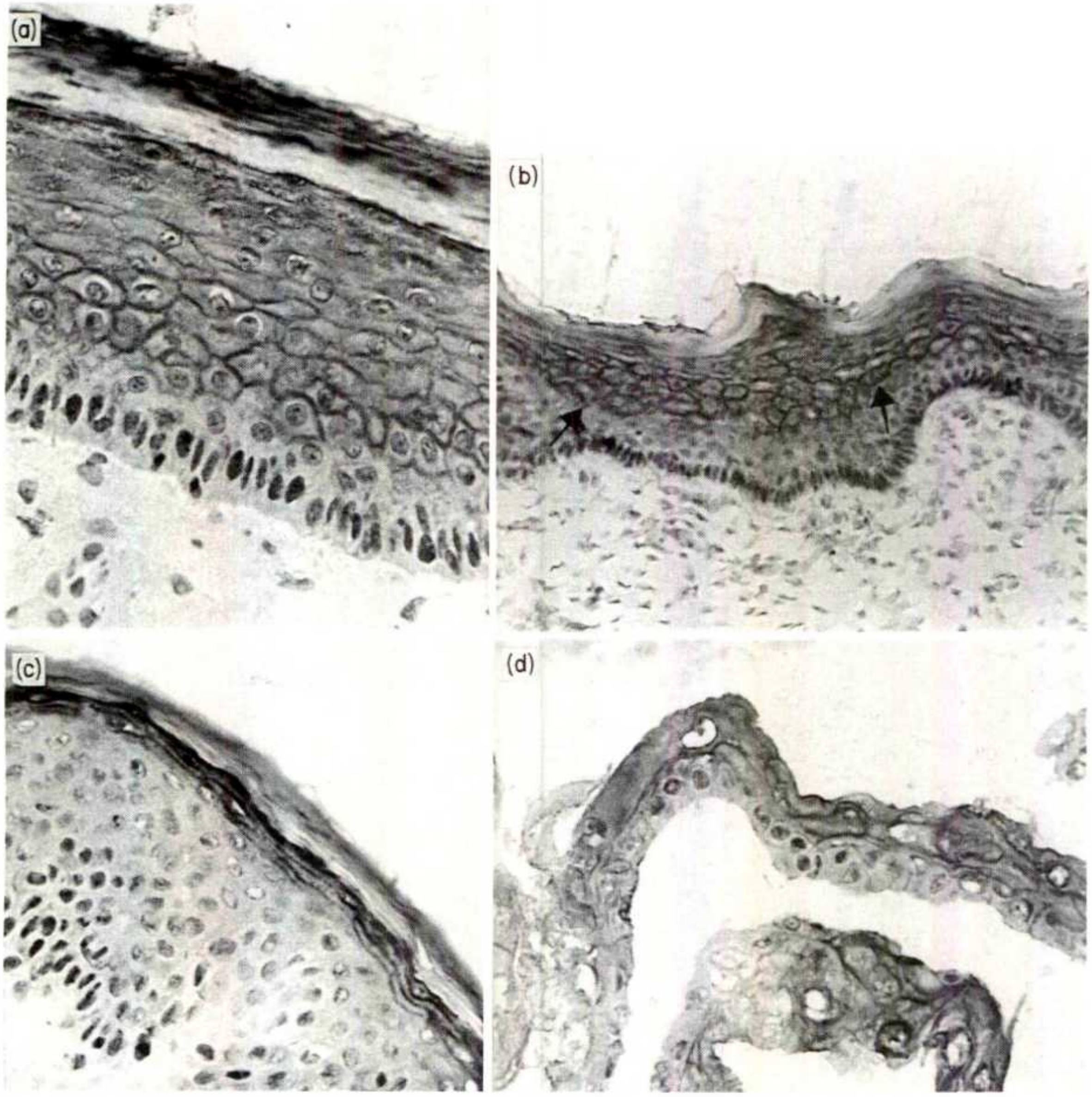

Figure 3. Pattern of involucrin expression in (a) keratinocyte allograft-treated skin 2 weeks post-grafting, showing involucrin around suprabasal cells (× 400); (b) keratinocyte allograft-treated burn 10 weeks post-grafting, showing that only the upper suprabasal cells are positive (arrows) (× 200); (c) normal skin, showing positive top layer (× 400); and (d) cultured epithelial sheet showing darkly stained suprabasal cells (× 400).

epidermis in the first few weeks of healing at allografted sites. The early absence of cytokeratin 10 does not agree with other observations,[20,21,23] although it has been shown to be reduced in regenerating skin.[19] However this may be due to the different antibodies used, in one case AE1 that recognizes a number of acidic keratins,[23] or the type of wound studied.[20,21]

The pattern of involucrin expression is of particular interest. Involucrin is the precursor of the cross-linked envelope protein of stratum corneum that forms during terminal differentiation of the keratinocyte.[25] In keratinocyte allograft sites up to 15 weeks post-grafting, involucrin was expressed throughout the suprabasal keratinocytes as opposed to only the granular layer in normal skin. Similarly at allograft treated ulcer and tattoo sites[26] and tape-stripped skin[19] involucrin was observed in the mid-epidermis, with normal distribution occurring 18 weeks after healing at allograft-treated ulcer sites.[21] This would indicate that the epidermis may take up to 4 months to differentiate fully into separate epithelial layers, and that histological examination alone is not enough. Involucrin expression is also increased in psoriasis[27] and squamous cell carcinoma[28] where the epidermal maturation is disrupted.

In summary, it is possible that keratinocyte allografts release cytokines such as TGFα to promote growth at wound sites and that initially these factors influence keratinocyte antigenic expression. Although epithelialization may be complete after 21 days, the newly formed epidermis remains in a highly proliferative and undifferentiated state for a number of months at epithelial allograft sites. The exact mechanism of action of these cultured allografts however has yet to be elucidated.

Acknowledgments

The authors would like to thank Mrs Irene Watson for typing the manuscript, and all the patients who donated biopsy material. This work was supported by the Grampian Health Board and Peel Medical Research Trust.

References

1 Hancock K, Leigh IM. Cultured keratinocytes and keratinocyte grafts. *Br Med J* 1989: **299:** 1179–80.

2 De Luca M, Albanese E, Bondanza S *et al.* Multicentre experience in the treatment of burns with autologous and allogenic cultured epithelium, fresh or preserved in a frozen state. *Burns* 1989; **15:** 303–9.

3 Leigh IM, Purkis PE, Navsaria HA, Phillips TJ. Treatment of chronic venous ulcers with sheets of cultured allogenic keratinocytes *Br J Dermatol* 1987; **117:** 591–7.

4 Burt AM, Pallett CD, Sloane JP *et al.* Survival of cultured allografts in patients with burns assessed with probe specific for Y chromosome. *Br Med J* 1989, **298:** 915–17.

5 Brain A, Purkis P, Coates P *et al.* Survival of cultured allogeneic keratinocytes transplanted to deep dermal bed assessed with probe specific for Y chromosome. *Br Med J* 1989; **298:** 917–19.

6 Moll R, Franke WW, Volc-Platzer B, Krepler R. Different keratin polypeptides in epidermis and other epithelia of human skin: a specific cytokeratin of molecular weight 46,000 in epithelia of the pilosebaceous tract and basal cell epitheliomas. *J Cell Biol* 1982, **95:** 285–95.

7 Mischke D, Wild G. Polymorphic keratins in human epidermis *J Invest Dermatol* 1987; **88:** 191–7.

8 de Mare S, Van Erp PEJ, Van de Kerkhof PCM. Epidermal hyperproliferation assessed by the monoclonal antibody KS 8.12 on frozen sections. *J Invest Dermatol* 1989; **92:** 130–1.

9 Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinising colonies from single cells. *Cell* 1975; **6:** 331–44.

10 Green H, Kehinde O, Thomas J Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. *Proc Natl Acad Sci USA* 1979: **76:** 5665–8.

11 Holmes JP, Rayner CRW. The technique of late dermabrasion for deep dermal burns: Implications for planning treatment *Burns Incl Thermal Inj* 1984, **10:** 349–54.

12 Compton CC, Gill JM, Bradford DA *et al.* Skin regenerated from cultured epithelial autografts on full thickness burn wounds from 6 days to 5 years after grafting. *Lab Invest* 1989; **60:** 600–12

13 Faure M, Mauduit G, Schmitt D *et al.* Growth and differentiation of human epidermal cultures used as auto and allografts in humans. *Br J Dermatol* 1987; **116:** 161–70.

14 Eisinger M, Sadan S, Silver IA, Flick RB. Growth regulation of skin cells by epidermal cell-derived factors: implications for wound healing. *Proc Natl Acad Sci USA* 1988; **85:** 1937–41.

15 Schultz GS, White M, Mitchell R *et al.* Epithelial wound healing enhanced by transforming growth factor-α and vaccinia growth factor. *Science* 1987, **235:** 350–1.

16 Coffey RJ Jr, Derynck R, Wilcox JN *et al.* Production and auto-induction of transforming growth factor-α in human keratinocytes. *Nature* 1987; **328:** 817–20.

17 Mansbridge J, Knapp M. Penetration of Lucifer yellow into human skin. A lateral diffusion channel in the stratum corneum. *J Invest Dermatol* 1988, **90:** 584.

18 Holland DB, Wood EJ, Cunliffe WJ, Turner DM. Keratin gene expression during the resolution of psoriatic plaques: effect of dithranol, PUVA, etretinate and hydroxyurea regimens *Br J Dermatol* 1989; **120:** 9–19.

19 Mansbridge JN, Knapp AM. Changes in keratinocyte maturation during wound healing. *J Invest Dermatol* 1987; **89:** 253–63.

20 Phillips TJ, Bhawan J, Leigh IM *et al.* Cultured epidermal allografts: Clinical use, survival and expression of maturation markers. *J Invest Dermatol* 1988; **90:** 598.

21 Phillips TJ, Bhawan J, Leigh IM *et al.* Cultured epidermal autografts and allografts. a study of differentiation and allograft survival. *J Am Acad Dermatol* 1990; **23:** 189–98.

22 Leigh IM, Purkis PE, Nausaria H. Cultured epithelium in the treatment of chronic leg ulcers and plastic surgery patients. In: *Proceedings of the International Symposium on Clinical Use of Cultured Epithelium in Surgery and Dermatology*. London, March 1987. Medical and Scientific Conferences Ltd, 1988.

23 Petersen MJ, Lessane B, Woodley DT. Characterisation of cellular elements in healed cultured keratinocyte autografts used to cover burn wounds. *Arch Dermatol* 1990, **126:** 175–83.

24 Oliver AM. The cytokeratin expression of cultured human foetal keratinocytes. *Br J Dermatol* 1990; **123:** 707–16.

25 Watt FM. Keratinocyte cultures: an experimental model for studying how proliferation and terminal differentiation are co-ordinated in the epidermis. *J Cell Science* 1988; **90:** 525–9.

26 Phillips T, Leigh IM, Purkis PE, Nausaria H. Expression of keratin polypeptides and histocompatibility antigens in wounds treated with allografts of cultured keratinocytes. *J Invest Dermatol* 1986; **87:** 161.

27 Murphy G, Flynn T, Rice R, Pinkus G. Involucrin expression in normal and neoplastic human skin: a marker for keratinocyte differentiation. *J Invest Dermatol* 1984; **82:** 453–7.

28 Said JW, Sassoon AF, Shintaku P, Banks-Schlegel S. Involucrin in squamous and basal cell carcinomas of the skin: An immunohistochemical study. *J Invest Dermatol* 1984; **82:** 449–52.

British Journal of Dermatology (1991) **125**, 155–163.

ADONIS 000709639100186F

Platelet and coagulation studies in Ehlers–Danlos syndrome

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Accepted for publication 2 February 1991

Summary

Fifty-one patients with Ehlers–Danlos syndrome were investigated for abnormalities of platelets and coagulation. Thirty-eight were examined prospectively and 13 retrospectively. A bleeding history was taken from all patients; only four (8%) gave no history of a bruising or bleeding tendency. Nine patients (18%) had significant haemostatic abnormalities of whom four (8%) had a platelet release defect, three (6%) had a factor XI deficiency and two (4%) had a factor XIII deficiency. Additionally 16 patients (31%) had mild abnormalities of uncertain significance of whom four (8%) had prolonged bleeding times (three in association with platelet aggregation abnormalities), 13 (26%) had platelet aggregation abnormalities and two had a positive Hess test. Twenty-four patients (47%) had normal tests for haemostasis of whom 20 (39%) had a bleeding diathesis and four (8%) had no such tendency. Results were analysed according to the type of Ehlers–Danlos syndrome, but there was no pattern to the abnormalities. The high frequency of a bleeding tendency in Ehlers–Danlos patients with normal tests for haemostasis (83%) supports the conventional explanation for this clinical feature, that defects in the structural integrity of skin and blood vessels lead to easy bruising.

The Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable disorders of connective tissue (Table 1).[1] The cardinal manifestations are hyperextensible soft velvety skin (Fig. 1), dystrophic scarring, joint hypermobility, connective tissue fragility and a bleeding tendency, mainly subcutaneous haematoma. The bleeding problem may be very severe and present with easy bruising (Figs 2 and 3), purpura, bleeding from gums after dental extraction (Fig. 4), gastrointestinal bleeding and haemoptyses. Rupture of major arteries is seen in type IV EDS and presents as an acute emergency.

The specific biochemical defects responsible for the various phenotypes of EDS are being identified. Type X EDS occurred with defective fibronectin in a kindred with a mild, recessively inherited variant of EDS in which a platelet aggregation defect segregated concordantly with skin and joint abnormalities.[2] Investigators have looked for an underlying abnormality to explain the bruising diathesis in other types of EDS, but studies to date have produced small patient numbers, brief haemostatic data and inconsistent findings (Table 2). We have therefore undertaken a larger study of patients with EDS to investigate haemostatic abnormalities and their correlation with clinical bleeding problems and EDS phenotype.

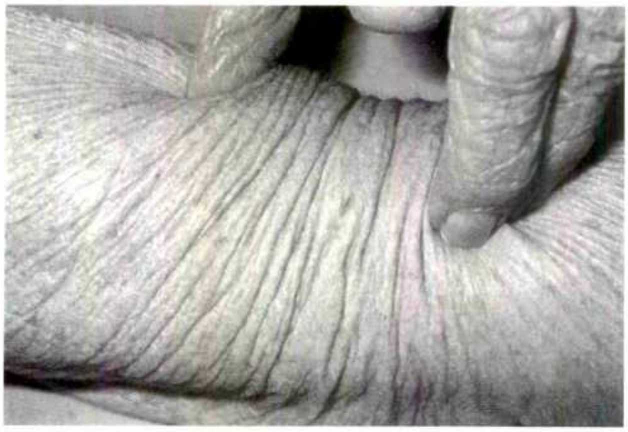

Figure 1. Soft, hyperextensible skin in type I Ehlers–Danlos syndrome.

Methods

Patients

Fifty-one patients with EDS were recruited from two dermatology centres (Wycombe General Hospital and Northwick Park Clinical Research Centre) and from the

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Table 1. Classification of Ehlers–Danlos syndrome[1]

Type		Inheritance	Clinical features
EDS I	Gravis type	AD	Cardinal manifestations in severe degree
EDS II	Mitis type	AD	Cardinal manifestations in mild degree
EDS III	Hypermobile type	AD	Marked articular hypermobility. Moderate dermal hyperextensibility. Minimal scarring
EDS IV	Vascular	Heterogeneous	Variable stigmata. Severe bruising. Hyperpigmentation and/or scarring. Vascular rupture. Colonic perforation. Characteristic facies
	IV-A Acrogeric type	AD	
	IV-B Acrogeric type	AR	
	IV-C Ecchymotic type	AD	
	IV-D Others	AD	
	(All forms have defective type III collagen)		
EDS V	X-linked	XL	Cardinal manifestations in moderate degree
EDS VI	Ocular-scoliotic type VI-A Decreased lysyl hydroxylase activity (VI-B Normal lysyl hydroxylase activity)	AR	Cardinal manifestations in severe degree Eye involvement. Scoliosis
EDS VII	Arthrochalasis multiplex congenita	Heterogeneous	Cardinal manifestations with marked articular hypermobility. Short stature. Micrognathia
	VII A structural defect pro-α	AD	
	VII B structural defect pro-α	AD	
	(VII C procollagen N-Proteinase deficiency?)	AR	
EDS VIII	Peridontitis type	AD	Cardinal manifestations in moderate degree Aggressive periodontitis, gingival recession Early tooth loss
EDS IX	Vacant (Formerly occipital horn syndrome)		
EDS X	Fibronectin abnormality	AR	Cardinal manifestations but skin texture normal. Petechiae. Striae distensae. Platelet aggregation defect corrected by fibronectin.
EDS XI	Vacant (formerly familial joint instability)		

Oxford Regional Haemophilia Centre. There were 20 males and 31 females with a mean age of 22 years (range 9–63 years). Thirty-eight patients were investigated prospectively and the results of 13 patients were analysed retrospectively. The 51 patients included 17 with type I EDS, 20 with type II EDS, five with type III EDS, seven with type IV EDS and two with type VIII EDS.

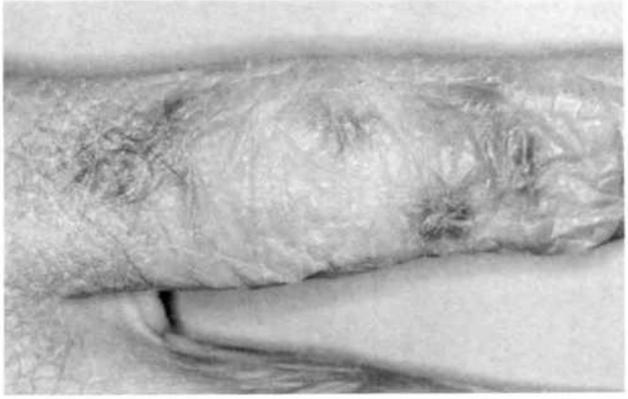

Figure 2. Small petechiae on the fingers in type I Ehlers–Danlos syndrome.

Haematology departments

Tests for haemostasis were performed at three haematology departments participating in this study. In addition a fourth haematology department (Frimley Park Hospital) investigated the kindred with EDS III and factor XI deficiency (Patients 26–29). The retrospective group were recruited from the Oxford Regional Haemophilia Centre which did not contribute any further patients to this study.

The normal ranges quoted are from the laboratory responsible for investigating 55% of the patients. There were minor variations in the normal ranges for tests of haemostasis between the four haematology departments but these were not relevant to the abnormalities identified in this study. All platelet aggregation studies used normal controls. The method of measuring bleeding time was standardized for Patients 1–38 (Ivy's method).

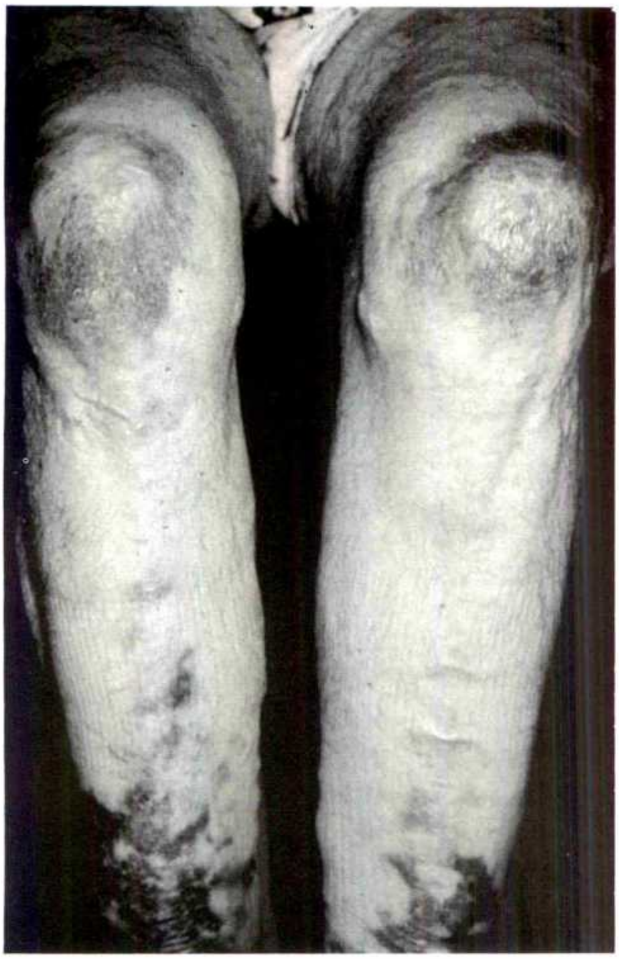

Figure 3. Extensive bruising over knees and lower legs in type I Ehlers–Danlos syndrome.

The method of measuring bleeding time in the retrospective group is uncertain but there were no abnormal or borderline results in this group.

Haemostasis

Screening tests of haemostasis were performed including the bleeding time (Ivy's method), Hess capillary fragility test, whole blood clotting time, partial thromboplastin time with kaolin, one-stage prothrombin time, prothrombin–consumption index, thrombin time, fibrinogen titre, platelet count (automated electronic method) and examination of a stained blood film. Platelet aggregation was performed by standard methods using the following agonists: collagen diluted in buffer to 40 μg/ml and 20 μg/ml solutions; adenosine diphosphate (ADP) diluted in saline to 100 μmol/l, 50 μmol/l, 25 μmol/l and 10 μmol/l solutions; arachidonic acid dissolved in 0·1 mol/l Na_2CO_3 to produce a 0·50 mmol/l solution; ristocetin sulphate diluted in saline to produce 15 mg/ml, 12 mg/ml and 10 mg/ml solutions; adrenaline diluted in 0·1% sodium metabisulphide to produce 20 μmol/l and 200 μmol/l solutions. Further investigations involved quantitative assay of one or more coagulation factors including factors XIII, XI and VIII VWF. Control tests using normal individuals were performed throughout. The methods used to investigate the haemostatic mechanism are fully described in Dacie and Lewis.[24]

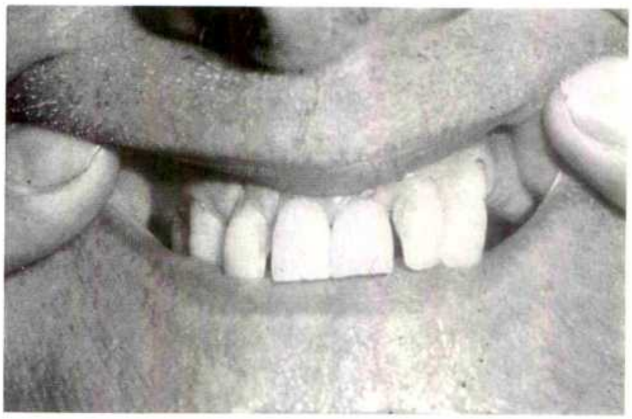

Figure 4. Periodontitis in type VIII Ehlers–Danlos syndrome, which may cause gingivitis and bleeding of the gums.

Results

The results of the investigations are shown in Table 3.

Platelet count, bleeding time and Hess capillary fragility test

The platelet count was normal (150–400 × 10^9/l) in all patients except in one where it was only marginally reduced. The bleeding time was performed on 37 patients and was prolonged in seven (range: 7·5 min to > 15 min). Of these seven patients, two (Numbers 13 and 31) had normal platelet aggregometry. One patient (Number 4) had a mildly prolonged bleeding time in association with a decreased response to ADP and arachidonic acid. The longest bleeding time was obtained from a patient (Number 30) in whom there was a markedly reduced response to collagen and adrenaline consistent with a platelet release defect. In two patients (Numbers 36 and 37) the bleeding time was moderately prolonged in association with a decreased response to ristocetin. The Hess capillary fragility test was performed in seven patients and was positive in two. Both patients

Table 2. Publications on platelet and coagulation abnormalities in Ehlers–Danlos syndrome

Authors		Reference	Number of patients in study	Coagulation	Platelets	Conclusion of study
Frick PG *et al.*	1956	3	3	Three had prolonged clotting times		
Day JH and Zarafonetis CJD	1961	4	4	Normal	Bleeding times normal	No platelet or coagulation abnormality identified
Goodman RM *et al.*	1962	5	9	PT prolonged in seven	CRT moderate in three and poor in three cases BT prolonged in three cases	A defect in platelets or other haemostatic factors could not be excluded
Kashiwagi H *et al.*	1965	6	3	PCI reduced in three cases TGT reduced in two cases	CFT increased in one case BT prolonged in one case	Platelet abnormality may contribute to bleeding dyscrasia in EDS
Barabas GM and Barabas AB	1967	7	5	Normal	CFT positive in all five cases	No clotting defects were identified
Estes JW	1968	8	3	Not done	Platelet aggregation studies mildly abnormal in two patients	Morphological and functional platelet abnormalities discussed
Beighton P *et al.*	1969	9	13	PT and PTT normal	Not done	No coagulation abnormality demonstrated
Hedner U and Nilson IM	1975	10	4	Not done	BT prolonged in three cases	Collagen suspensions from all 4 patients aggregated platelets less effectively than controls
Arneson MA *et al.*	1980	2	4		Platelet aggregation abnormalities reversed by addition of fibronectin	Fibronectin deficiency accounts for both the features of EDS and the platelet abnormalities
Various case reports	1960–1989	11–23	16	XIII deficiency VIII deficiency IX C and IX R·AG mild deficiency	Various abnormalities of platelets have been identified	
Total			64			

PT, prothrombin time; PTT, partial thromboplastin time; PCI, prothrombin consumption index, TGT, thromboplastin generation time, CRT, clot retraction test; BT, bleeding time; CFT, capillary fragility test.

(Numbers 44 and 49) had a normal platelet count and bleeding time; in neither case was platelet aggregometry performed.

Platelet aggregation studies

Platelet aggregometry was normal in 22 out of 40 patients investigated. Isolated defects in aggregation occurred in one patient (Number 4) to collagen, in five patients (Numbers 5, 6, 9, 16 and 42) to ADP affecting both primary and secondary responses, and in five patients (Numbers 19, 34, 35, 36, 37) to ristocetin. The five patients with an isolated absence of response to ristocetin had normal coagulation screens. No patients

Table 3. Platelet and coagulation studies in Ehlers–Danlos syndrome

Patient no	Sex	Type of EDS	Platelets	Bleeding time (min/sec) ($n=1$–7 min)	Platelet aggregometry					Factor XIII	Clotting screen	Comments
					Collagen	ADP	Arachidonate	Ristocetin	Adrenaline			
Prospective group												
1	F	I	204	5.45	↑	N	—	N	—	N	N	Bruising tendency
2	M	I	225	6.10	Abs	↓	Abs	↓	—	N	N	Platelet release defect. Bruising & bleeding tendency
3	M	I	241	—	↓	↓	↓	↓	—	N	N	Platelet release defect. Sibling Case 2 Bruising tendency
4	F	I	230	7.30	↓	N	N	N	—	N	N	Bruising tendency
5	M	I	228	—	N	↓	N	N	—	N	N	Bruising and bleeding tendency
6	F	I	178	2.00	N	↓	N	N	—	N	N	Mild bruising tendency
7	M	I	261	4.50	↓	↓	N	↓	—	N	N	Platelet release defect Bruising tendency
8	M	I	390	6.30	↓	↓	N	↑	—	N	N	Mild bruising tendency
9	F	I	292	4.30	↑	↓	N	↑	—	N	N	Recurrent bruises, particularly on legs
10	F	I	311	2.44	N	N	—	—	—	N	N	Mild bruising tendency
11	M	I	235	7.00	N	N	N	N	N	N	N	Recurrent troublesome bruising hands and feet
12	M	I	246	—	N	N	—	N	N	N	N	Son of Case 11 Bruises very easily
13	F	I	344	13.30	N	N	—	N	N	N	N	Bruising tendency
14	F	II	403	5.00	N	N	—	—	—	N	N	Mild bruising tendency
15	M	II	423	5.00	N	N	—	—	—	N	N	Sibling of Case 14 Bruising tendency
16	F	II	351	5.55	N	↓	N	N	—	N	N	Mild bruising tendency
17	M	II	252	8.00	N	↓	↓	—	—	1–2%	Prolonged	Marked bruising and bleeding
18	F	II	284	5.30	N	N	↓	Abs	—	N	N	Bruising tendency
19	F	II	230	4.15	N	N	N	↓	—	1–2%	N	Bruising and bleeding troublesome and recurrent.
20	F	II	284	6.30	N	N	N	N	—	—	N	Severe nose bleeds
21	M	II	272	6.00	N	N	N	N	—	N	N	Sibling of Case 20. Marked bruising tendency
22	F	II	233	—	N	N	N	N	—	—	N	Bruising tendency Heavy bleed post tonsillectomy
23	F	II	310	6.30	N	N	N	N	—	—	N	Mother of Case 22. Mild bruising tendency
24	M	II	214	6.00	N	N	N	N	—	N	N	Mild bruising tendency
25	F	III	270	6.45	N	N	—	N	N	N	N	Recurrent bruising on forearms
26	F	III	N	—	—	—	—	—	—	—	Prolonged KCCT	Factor XI Defic History of bruising. Mother of cases 27,28,29
27	M	III	N	5.30	—	—	—	—	—	—	Prolonged KCCT	Factor XI Defic Bruising tendency
28	F	III	N	—	—	—	—	—	—	—	Prolonged KCCT	Factor XI Defic.
29	F	III	N	—	—	—	—	—	—	—	Prolonged KCCT	Factor XI normal
30	F	IV	270	>15 min	↓	↓	↓	Abs	—	N	N	Platelet release defect Bruising tendency
31	F	IV	N	8.00	N	N	N	N	—	—	N	Bruising tendency
32	M	IV	N	7.00	N	N	N	N	—	—	N	Bruising and bleeding tendency
33	F	IV	N	—	N	N	N	N	—	—	N	Sibling of Case 32 Troublesome bruising
34	M	IV	225	3.10	N	N	N	Abs	—	N	N	Recurrent large bruises
35	M	IV	300	6.00	N	N	N	↓	—	N	N	Sibling of Case 34 Marked bruising
36	M	IV	262	10 00	N	N	N	↓	—	N	N	Bruising and bleeding tendency
37	F	VIII	285	12.00	N	N	N	↓	—	N	N	Mild bleeding tendency from gums
38	M	VIII	232	6.30	N	N	—	N	—	N	N	No bruising/bleeding tendency
Retrospective group												
1	M	I	294	—	N	N	N	N			N	Easy bruising since age 2
2	F	I	N	6.00	N	N	N	N			N	Easy bruising Post op bleeding
3	F	I	353	3 30	N	N	N	N			N	Menorrhagia. Excessive bruising
4	F	I	252	4 45	N	↓	N	N			N	Easy bruising
5	M	II	143	3 22	N	N	N	N			—	Easy bruising Father of Case 6
6	F	II	210	6 00	N	N	N	N			—	Easy bruising Daughter of Case 5
7	F	II	231	2 03	—	—	—	—			—	Easy bruising Pos tourniquet test
8	F	II	N	2 50	—	—	—	—			—	No bleeding problems Hess test neg.
9	M	II	N	1.30	—	—	—	—			—	Son of Case 8. Hess test neg Easy bruising
10	F	II	N	2 15	—	—	—	—			—	No bleeding problems Hess test neg
11	F	II	N	5.45	—	—	—	—			—	No bleeding problems Hess test neg.
12	F	II	N	2 00	—	—	—	—			—	Hess test weak positive Bruising tendency
13	F	II	N	4 00	—	—	—	—			—	Hess test negative Bruising tendency Cases 10–13 Family study

↑, increased, ↓, decreased; Abs, absent, —, not done, N, normal

Table 4. Haematological abnormalities according to EDS type

EDS type	Number of cases	Platelets/bleeding	Clotting studies and coagulation factor assays
I	17	Three cases of platelet release defect Seven cases with isolated aggregation abnormalities	No abnormality identified
II	20	Four cases with isolated aggregation abnormalities	Two cases of factor XIII deficiency
III	5	No abnormality identified	KCCT prolonged in a kindred of four; Factor XI assay low/normal in one case and deficient in two cases
IV	7	One case of platelet storage pool disease Three cases with reduced or absent platelet aggregation to ristocetin	No abnormality identified
VIII	2	One case with prolonged bleeding time and reduced platelet aggregation to ristocetin	No abnormality identified

showed an isolated aggregation deficiency with arachidonate. A pattern of reduced response occurred with collagen and ADP with primary response only (Number 8), ADP (absent primary and secondary responses) and arachidonic acid (Number 16) and arachidonic acid and ristocetin (Number 18).

There was a reduced response to collagen and adrenaline compatible with platelet release defect in four patients (Numbers 2, 3, 7 and 30). Of these four patients, one (Number 30) had a markedly prolonged bleeding time; the other three patients had normal bleeding times.

Coagulation screen and clotting factor assays

A one-stage prothrombin time and partial thromboplastin time with kaolin was performed in 42 patients, and was normal in 37. A family of four with EDS type III all had a prolonged partial thromboplastin time. Three of these four patients had a factor XI deficiency (normal range 50–150 IU/dl), with factor XI levels of 38 IU/dl (Number 27), 34 IU/dl (Number 28) and 33 IU/dl (Number 26).

Factor XIII was assayed in 26 patients and was normal in 24. In two (Numbers 17 and 19) a marked factor XIII deficiency was identified (1–2% of normal). In Patient 17 there was also a mildly prolonged bleeding time; the prothrombin time was mildly prolonged at 15 s (control 12 s) and the partial thromboplastin time with kaolin prolonged at 51 s (control 39 s). In Patient 19 the bleeding time and coagulation screen were normal, but there was reduced platelet aggregation to ristocetin.

Haematological abnormalities according to EDS type

The results are shown in Table 4. Type I EDS was represented by 13 patients in the prospective group and four patients in the retrospective group. No coagulation defect or factor XIII deficiency was identified. Platelet aggregation abnormalities were present in 10 patients and were normal in seven. Three of the four cases of a platelet release defect identified in our study overall occurred in type I EDS.

Type II EDS included 11 patients in the prospective group and nine in the retrospective group. Both cases of factor XIII deficiency occurred in type II EDS; no other coagulation abnormality was present in the nine other patients investigated prospectively. Platelet aggregation was normal in nine patients and abnormal in four, with isolated abnormalities only, and no cases of platelet release defect were identified.

The five cases of type III EDS included a family of four all with a prolonged partial thromboplastin time of whom three had low levels of factor XI. Platelet aggregation studies were performed in one case and were normal.

Type IV EDS was represented by seven patients who were all investigated prospectively. No coagulation abnormality was present. Platelet aggregation studies identified one patient with a platelet release defect disease and three patients with reduced or absent response to ristocetin.

In the two patients with type VIII EDS no abnormality in haemostasis was found in one case; the second patient had a prolonged bleeding time and reduced platelet aggregation with ristocetin.

Discussion

Numerous papers on haemostatic abnormalities in EDS have been published but no investigator has yet analysed a substantial series of these patients in an attempt to correlate laboratory findings with clinical bleeding problems and the EDS phenotype. The usual explanation for the bleeding diathesis in EDS is an abnormal capillary structure with a deficiency of perivascular collagen producing a tendency for poorly supported cutaneous blood vessels to be ruptured when subjected to shearing forces. This does not explain the wide diversity in bleeding seen in EDS that ranges from mild bruising in types II and III EDS to severe and sometimes fatal bleeding in type IV EDS, and a tendency to intraocular haemorrhage in type VI EDS.

It is possible that the identification of haemostatic abnormalities will have a greater significance than is initially apparent, as demonstrated by the identification of type X EDS[2]. A haematological separation of sporadic male cases of type II EDS and type V EDS, which have similar clinical features, could be useful for genetic counselling.

Platelet abnormalities in EDS have been reported and include a variety of functional and morphological defects; as in our study there are no reports of significant associated thrombocytopenia. In patients with platelet dysfunction, the bleeding time may be prolonged due to inability of the platelets to form aggregates normally. Seven of 37 patients in our study had prolonged bleeding times. Day and Zarafonetis[4] reported normal bleeding times in four patients studied. A prolonged bleeding time has been reported in various studies: in three out of nine patients,[5] in one out of three patients,[6] in three out of four patients[10] and by Caen *et al.*[14] in a single case report which included impaired response in platelet aggregation to adrenaline and ADP. The Hess capillary fragility test may be positive in disorders affecting either platelets or the capillary wall. Two of seven patients tested in our series had positive Hess tests. Few other studies have included Hess tests: Barabas and Barabas[7] reported five patients with positive Hess tests, of whom only one gave a history of easy bruising.

Platelet aggregation studies are an essential part of the investigation of patients with suspected platelet dysfunction. A variety of platelet aggregation abnormalities has been found in our patients, ranging from isolated defects to aggregation reagents, to a more generalized impairment of response associated in some cases with a prolonged bleeding time. Four patients were identified as having platelet aggregometry results compatible with a platelet release defect. The EDS literature cites many results of platelet aggregation studies; most reports showed normal[13,17,19] or mildly abnormal platelet aggregation.[8,16] Collagen suspensions prepared from EDS patients (type not specified) produced poor platelet aggregation in two patients reported by Karaca *et al.*[13] and four patients reported by Hedner and Nilson;[10] however, a similar study by Deliyannis *et al.*[17] produced normal platelet aggregation.

Abnormalities in platelet aggregation helped to identify the underlying abnormality of type X EDS.[2] A family with a mild, recessively inherited variant of EDS was reported in which defective platelet aggregation induced by collagen was corrected by fibronectin-rich cryoprecipitate. It was proposed that both the bleeding disorder and joint hypermobility were likely to be explained by qualitatively defective fibronectin (dysfibronectinaemia).

Estes *et al.*[25] reported five patients with Marfan's syndrome, easy bruising and giant platelets, four of whom had functional platelet defects. This chance discovery led to a more substantial investigation of platelet and coagulation abnormalities in the connective tissue diseases Marfan's syndrome, osteogenesis imperfecta, EDS, pseudoxanthoma elasticum and a group of mucopolysaccharidoses.[8] Two cases of EDS (type unspecified) were included in this series of 29 patients, of whom 15 were studied using electron microscopy; no platelet ultrastructural abnormalities were identified. Two of three patients with EDS (type unspecified) reported by Kashiwagi *et al.*[6] had a severe haemorrhagic diathesis; all three patients showed consistent and marked platelet ultrastructural changes, including a decrease in the number of dendritic forms and an increase in abortive dendritic and spread forms; platelets from the four children of one of the index cases revealed similar ultrastructural abnormalities.

Five patients in our series had an isolated impaired platelet aggregation to ristocetin (Numbers 19, 34, 35, 36 and 37). This pattern may occur in von Willebrand's disease or Bernard Soulier syndrome, a condition characterized by giant platelets.[26] Our five patients had a normal partial thromboplastin time and platelet size. The findings in von Willebrand's disease can be very variable and this diagnosis cannot be excluded in our patients. We did not have the opportunity to perform factor VIII studies which are necessary to confirm or refute this diagnosis.

Platelet aggregation with arachidonic acid was deficient in one patient (Number 18) in association with absence of aggregation with ristocetin. An impaired response to arachidonic acid, collagen and adrenaline is seen in cyclo-oxygenase or thromboxane synthetase deficiency and as an 'aspirin defect'. No patient in our study was taking aspirin-like drugs.

Coagulation defects reported in EDS are rarer than platelet abnormalities. Some early reports of prolonged coagulation in EDS did not include attempts to identify a coagulation factor deficiency.[4,5] Deficiencies in clotting factors VIII,[18,22] IX[11,20] and XII[12] have now been reported in association with EDS, but were not identified in our series. A single case of EDS occurring in association with factor XIII deficiency has been reported.[21] We have found two patients with an associated factor XIII deficiency. Factor XIII deficiency is a rare autosomal recessive disorder which is characterized by a severe haemorrhagic tendency,[27] and in common with EDS, poor wound healing with prominent scar formation and pseudotumours. The factor XIII-α chain is absent from plasma and platelets and this results in friable fibrin clots due to the absence of cross-linking. Congenital factor XIII deficiency is rare[28] with only about 100 cases reported in the literature, and the three cases of factor XIII deficiency in EDS now identified may amount to more than a chance association.

To our knowledge, five clotting factor deficiencies have now been reported in association with EDS. Defects at a single genetic locus cannot account for both the connective tissue changes in EDS and these clotting factor deficiencies. Genes for individual clotting factors are scattered throughout the human genome, with factors VIII and IX on the X-chromosome, factor XI on chromosome 4, factor XII on chromosome 5, factor XIII-a on chromosome 6 and factor XIII-b on chromosome 1. Regulation of genes is usually controlled by upstream 5′ or downstream 3′ promoters or enhancers and are part of the genes concerned; abnormalities of these regions would not explain multiple coagulation defects.

In conclusion, the high frequency of a bleeding tendency in EDS patients with normal tests for haemostasis supports the conventional explanation for this clinical feature. Haemostatic abnormalities identified in this series include four patients with a platelet release defect, two with factor XIII deficiency and three with factor XI deficiency. These abnormalities occurred more frequently than was expected. It is important in EDS patients to avoid elective surgery, because of multiple haemostatic defects, friable tissues and delayed wound healing.

Acknowledgments

The authors wish to thank Kate Anstey for preparing the manuscript, Dr. C.Rizza for allowing the use of data on patients from the Oxford Regional Haemophilia Centre and for helpful comments, Dr John Wilkinson for allowing his patients to be included in this series and Drs. M. Sharland and M. Patton for their helpful advice.

References

1 Beighton P, de Paepe A, Danks D *et al.* International nosology of heritable disorders of connective tissue, Berlin 1986. *Am J Med Genetic* 1988; **29:** 581–94.

2 Arneson MA, Hammerschmidt DE, Furcht LT, King RA. A new form of Ehlers–Danlos syndrome. Fibronectin corrects defective platelet function. *J Am Med Assoc* 1980; **244:** 144–7.

3 Frick PG, Krakohuk JO. Studies of haemostasis in the Ehlers–Danlos syndrome. *J Invest Dermatol* 1956; **26:** 453–7.

4 Day HJ, Zarafonetis CJD Coagulation studies in four patients with Ehlers–Danlos syndrome. *Am J Med Science* 1961; **242:** 565–73

5 Goodman RM, Levitsky JM, Friedman IA. The Ehlers–Danlos syndrome and multiple neurofibromatosis in a kindred of mixed derivation, with special emphasis on haemostasis in the Ehlers–Danlos syndrome. *Am J Med* 1962; **32:** 976–83.

6 Kashiwagi H, Riddle JM, Abraham JP, Frame B. Functional and ultrastructural abnormalities of platelets in Ehlers–Danlos syndrome. *Ann Intern Med* 1965, **63:** 249–54.

7 Barabas GM, Barabas AP. The Ehlers–Danlos syndrome. A report of the oral and haematological findings in nine cases. *Br Dent J* 1967; **123:** 473–9.

8 Estes JW. Platelet size and function in the heritable disorders of connective tissue. *Ann Intern Med* 1968; **68:** 1237–49.

9 Beighton P, Price A, Lord J, Dickson E. Variants of the Ehlers–Danlos syndrome. Clinical, biochemical, haematological, and chromosomal features of 100 patients. *Ann Rheum Dis* 1969; **28:** 228–45.

10 Hedner U, Nilson IM. Ehlers–Danlos syndrome. *Thrombos Diathes Haemorrh (Stuttg)* 1975; **33:** 380–1.

11 Lisker R, Nogueron A, Sanches-Medal L. Plasma thromboplastin component deficiency in the Ehlers–Danlos syndrome. *Ann Intern Med* 1960; **53:** 388–95.

12 Fantl P, Morris KN, Sawers RJ. Repair of cardiac defect in patient with Ehlers-Danlos syndrome and deficiency of Hageman factor. *Br Med J* 1961; **1:** 1202–4.

13 Karaca M, Cronberg L, Nilsson IM. Abnormal platelet—collagen reaction in Ehlers–Danlos syndrome. *Scand J Haematol* 1972; **9:** 465–9.

14 Caen J, Legrand Y, Sultan Y *et al.* Allongement du Temps de Saignement et anomalies du collagene. *Soc Francaise D'Hematologie* 1969; **10:** 426–30.

15 Estes JW. Platelet abnormalities in heritable disorders of connective tissues. *Ann NY Acad Med* 1972; **201:** 445–50.

16 Onel D, Ulutin SB, Ulutin ON. Platelet defect in a case of Ehlers–Danlos syndrome. *Acta Haematol* 1973; **50:** 238–44.

17 Deliyannis AA, Kontopoulou-Griva I, Tsevrenis HV. Normal platelet aggregating properties of Ehlers–Danlos syndrome 'collagen'. *Thrombosis et Diathesis Haemorrhagica* 1974, **32:** 203–6.

18 Clough V, MacFarlane A, O'Connor J, Wood JK. Acquired von Willebrand's syndrome and Ehlers–Danlos syndrome presenting

with gastrointestinal bleeding. *Scand J Haematol* 1979; **22:** 305–10.

19 Nanfroo JJ. Spontaneous rectus haematoma. Occurrence in a patient with Ehlers–Danlos syndrome type IV. *Arch Dermatol* 1982; **118:** 695–6.

20 Gamba G, Gatti V, Longoni P *et al.* Type IV Ehlers–Danlos syndrome and Factor IX deficiency: A case report. *Haematologica* 1986; **71:** 139–41.

21 Suzuki T, Tsuchiyama Y, Kauraguchi Y *et al.* Anaesthesia for a patient with Ehlers–Danlos syndrome and factor XIII deficiency. *Masui* 1989; **38:** 1518–21 (English abstract).

22 Bertin P, Treves R, Julia A *et al.* Ehlers–Danlos syndrome, clotting disorders and muscular dystrophy. *Ann Rheum Dis* 1989; **48:** 953–6.

23 Anstey A, Wilkinson JD, Pope FM. Ehlers-Danlos syndrome with recurrent bruising. *J Royal Soc Med* 1990; **83:** 800–1.

24 Dacie JV, Lewis SM. *Practical Haematology*, 6th edn. Churchill Livingstone, 1984; 213–222, 230–238, 249–253.

25 Estes JW, Carey RJ, Desai RG, Marfans syndrome: Haematological abnormalities in a family. *Arch Intern Med* 1965; **116:** 889–93.

26 Bernard J, Soulier JP. Sur une nouvelle variete dystrophie Thrombocytaire hemorrhagipare congenitale. *Semaine des Hopitaux Paris* 1948; **24:** 3217–23.

27 Losowsky MS, Miloszewski KJA. Factor XIII. *Br J Haematol* 1977; **37:** 1–4.

28 Lorand L, Urayama T, Atencio A, Hsia DYY. Inheritance of deficiency of fibrin stabilizing factor (factor XIII). *Am J Human Genet* 1970; **22:** 89–95.

British Journal of Dermatology (1991) 125, 164–168. ADONIS 000709639100187S

Alopecia areata in children: response to treatment with diphencyprone

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Accepted for publication 27 February 1991

Summary Twelve children with extensive alopecia areata or alopecia totalis were treated with the contact allergen diphencyprone. The duration of treatment ranged from 5 months to 1 year. Eight of the 12 (67%) regrew scalp hair and in four (33%) there was a complete regrowth. Six months after treatment was discontinued three of the four children with complete regrowth maintained their hair, one had lost all the regrowth and a further child with patchy regrowth at the end of treatment subsequently regrew hair completely while off therapy.

The contact allergen diphencyprone (DPC), has already been shown by some centres to be a useful agent in the treatment of alopecia areata (AA).[1–3] We report the results of treatment with DPC in 12 children with persistent AA or alopecia totalis (AT).

Methods

Diphencyprone was supplied by Kodak Laboratories and screened prior to use for the presence of the mutagenic precursor $\alpha\alpha_1$,dibromodibenzyl ketone.[4] Ethical committee approval was obtained for the trial in which 12 children, eight male and four female, were included. Their ages were in the range 5–15 years (mean 11 years) and duration of AA varied from 1 to 13 years (mean 4·4 years). Four of the 12 children presented with AA (two females, two males); the remaining eight (six males and two females) presented with AT. Informed consent was given by the children's parents. Prior to starting treatment, baseline haematological investigations were made which included a full blood count, hepatic and renal function, antinuclear factor, rheumatoid factor, thyroid antibodies and immunoglobulins. Side-effects that might develop were fully explained to both parents and children, including the development of a vesicular eruption in the area of treatment, cervical lymphadenopathy and exacerbation of existing eczema.

Diphencyprone is readily degraded by ultraviolet light[5] and therefore it was made up with acetone and stored in dark glass bottles. The patients were instructed to keep their heads covered for a minimum of 6 h post-therapy and requested not to wash their heads for 48 h.

Sensitization was initiated with a 2% solution applied to a small area on the scalp (4 cm diameter) and was achieved when the area became red and pruritic. If this reaction did not occur the procedure was repeated beneath elastoplast occlusion. Alternatively, if the patient experienced redness alone with no pruritus, or if a reaction developed within 24 h of the initial application, this was not considered to be an allergic reaction, and 2% DPC was reapplied with occlusion.

Once sensitized, a weak solution (0·01%) was applied to one side of the scalp, this was repeated weekly, the concentration of the DPC solution being gradually increased (0·025, 0·05, 0·075–2%) until the patient experienced erythema and pruritus for 36 h in the area of treatment. The concentration of DPC producing this degree of allergic reaction was considered to be the optimum concentration for stimulation of hair growth.[6] Once the optimum concentration of DPC solution for an individual was established, it was applied to the whole scalp. The applications were made weekly and were performed by a doctor or a nurse. Every patient was given a potent topical steroid cream (betamethasone valerate) for use in the event of a vesicular eruption developing in the area of treatment.

The patients and their parents were advised that treatment would continue for 8 months, and would be stopped if there was no hair growth after that time.

In previous work with DPC the protocol demanded that initially only one side of the scalp was treated, thereby establishing that any subsequent unilateral hair growth on the treated side was secondary to the application of DPC. In this way the ability of DPC to stimulate hair growth has already been demonstrated.[7] The authors felt that it was unreasonable to expect a child to tolerate unilateral hair growth and it was decided to treat the whole scalp simultaneously.

Results

All 12 children tolerated treatment with DPC without difficulty. The length of treatment varied from 5 months to 1 year (mean 7·3 months). Six of the 12 were atopic and eight had a family history of AA. None of the children demonstrated the presence of autoantibodies (Table 1).

The main side-effect was an exacerbation of mild eczema as seen in three of the cases (Patients 1, 6 and 7). Patient 1 developed infected eczema of the scalp requiring hospital admission and oral antibiotics. He quickly responded and continued treatment with DPC without any further problems. Patient 6 developed blistering of the scalp in the area beneath the rim of his cap; following modification of his headwear, this resolved. In each case the side-effects were insufficient to necessitate discontinuation of DPC treatment (Table 2).

Table 1. Patient details

Patient	AA/AT	Sex	Age	Duration of AA/AT (years)	Family history	Atopic	Antibodies
1	AT	M	8	3	–	+	–
2	AA	M	5	4	+	+	–
3	AT	M	10	3	–	–	–
4	AT	M	15	8	+	–	–
5	AT	F	14	6	+	–	–
6	AT	M	10	3	+	–	–
7	AA	F	14	1	–	–	–
8	AT	F	12	7	–	+	–
9	AA	F	13	1	+	+	–
10	AT	M	11	2	+	+	–
11	AA	M	14	13	+	–	–
12	AT	M	8	3	+	+	–

Table 2. Results of treatment

Patient	AA/AT	Sex	Duration of AA/AT (years)	Duration of DPC treatment (months)	Side-effect	Hair growth
1	AT	M	3	7	Infected eczema of scalp	—
2	AA	M	4	6	—	—
3	AT	M	3	5	—	Fine regrowth over whole scalp
4	AT	M	8	6	—	Regrowth of thick terminal hair over whole scalp
5	AT	F	6	8	—	Patchy regrowth
6	AT	M	3	5	Blistered where cap rubbed	—
7	AA	F	1	9	Eczema nape of neck	Patches all filled in
8	AT	F	7	12	—	Patchy regrowth
9	AA	F	1	8	—	Patches all filled in
10	AT	M	2	8	—	—
11	AA	M	13	6	—	Patchy regrowth
12	AT	M	3	8	—	(Fine) regrowth over whole head

Table 3. Children with no hair growth following treatment with DPC

Patient	AT/AA	Duration of AA (years)	Duration of treatment with DPC (months)
1	AT	3	7
2	AA	4	6
6	AT	3	5
10	AT	2	8

Hair growth

Four of the 12 patients (three AT; one AA) had no growth of scalp hair. These were all males, the duration of their alopecia varied from 2 to 4 years (mean 3 years) and the duration of treatment with DPC ranged from 5–8 months (mean 6·5 months) (Table 3).

Eight of the 12 patients showed some regrowth. Five of these presented with AT (three males, two females) and three with AA (one male, two females). The duration of

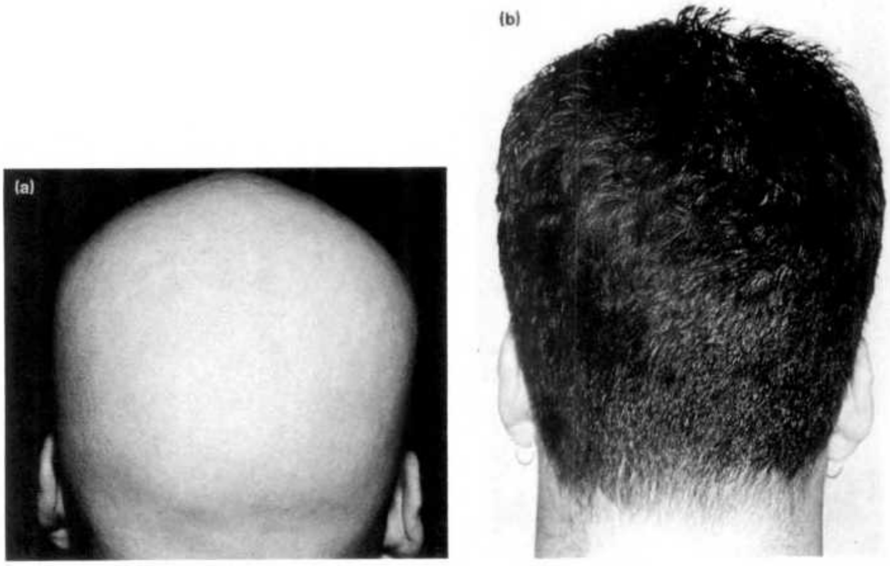

Figure 1. Patient 4 (a) before and (b) at the end of treatment.

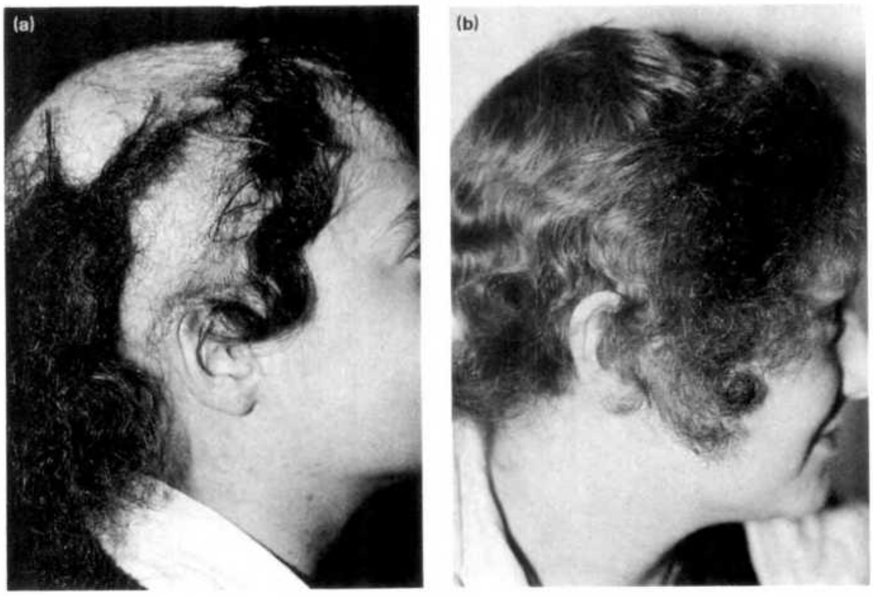

Figure 2. Patient 9 (a) before and (b) at the end of treatment.

Table 4. Children with hair growth

Patient	AA/AT	Duration of AA/AT (years)	Duration of DPC treatment (months)	Growth	Hair status 6 months after DPC discontinued
3	AT	3	5	Initial regrowth followed by hair fall	No change
4	AT	8	6	Complete regrowth of terminal hair	One patch
5	AT	6	8	Patchy regrowth	Some loss
7	AA	1	9	All patches regrown	No loss
8	AT	7	12	Patchy regrowth	Further growth
9	AA	1	8	All patches regrown	One patch
11	AA	13	6	Patchy regrowth	Some loss
12	AT	3	8	Growth of terminal hair over whole scalp	All regrown hair lost

alopecia varied from 1 to 13 years (mean 5·3 years) and the duration of treatment ranged from 5 to 12 months (mean 8 months). Four of the eight had complete regrowth, of whom two patients (4 and 12) had presented with AT and two (Patients 7 and 9) had presented with AA (Figs 1 and 2). One patient (3) regrew fine terminal hair over all the scalp, but subsequently lost it despite continuing treatment with DPC; and three patients (5, 8, 11) responded with patchy regrowth (Table 4).

Subsequent progress

Three patients (5, 8, 11) were showing patchy regrowth when the supply of DPC was withdrawn and treatment discontinued (Table 2). Subsequently, all patients were assessed at 2-monthly intervals for 6 months. During this period most patients, apart from those who had full regrowth of hair (Patients 4, 7, 9 and 12), were given a potent topical steroid cream or lotion (clobetasol propionate) to apply to the scalp or the remaining areas of hair loss, daily.

Of the eight patients with hair growth at the end of treatment, two (7 and 8) showed no loss of hair 6 months after cessation of DPC (Table 4). Patient 8, who had patchy regrowth at the end of treatment, went on to regrow a complete head of hair with no further therapy (topical steroids were declined).

Patients 4 and 9 developed one patch 6 months after the end of treatment; patient 4 responded quickly to further DPC when a supply was restablished. Patient 9 preferred to delay further treatment. Patient 12, who had a full regrowth of hair and was therefore given no topical steroid, lost all his regrowth within 2 months. Patients 5 and 11 experienced some loss despite the use of a topical steroid during this time, but did not reach their pretreatment level of hairlessness. Patient 3 lost all the regrowth while on therapy and, as yet, has not restarted treatment. None of the four patients who showed no growth of hair while being treated with DPC grew hair during the 6 months after treatment was withdrawn.

In summary, eight out of 12 children (67%) with extensive AA or AT regrew scalp hair following treatment with DPC. Four (33%) had complete regrowth. Six months after treatment was discontinued, four of the eight children with regrowth maintained a good, cosmetically acceptable, head of hair.

Discussion

The effectiveness of DPC in the treatment of AA in adults has been demonstrated previously.[1, 2, 3, 7] However, the use of DPC in the treatment of AA occurring in childhood has not previously been reported. Extensive AA or AT occurring in childhood is generally considered to be a more intractable disease than in adults. Nevertheless, the response to DPC in these children was very similar to that seen in our previous cohort of adult patients and 33% and 29%, respectively, achieved complete regrowth of hair.[7] Similarly, as in the adult study, the duration of alopecia appeared to have no effect on the outcome of treatment.

Because AA is considered to be an autoimmune disease, a familial tendency towards AA might be expected. In this study of childhood alopecia, eight (67%) had a positive family history, but this did not appear to be a detrimental feature with regard to the response to DPC, in that five of the eight with regrowth also had a positive family history. Similarly, atopy did not reduce the child's ability to respond to DPC, and three of six children who were atopic responded, two with complete regrowth.

Overall, this study has demonstrated that DPC is an effective treatment for AA of childhood, particularly as four of the eight with regrowth (33% of the patients) retained a cosmetically acceptable head of hair 6 months after treatment was discontinued.

However, treatment of AA with a contact allergen is not curative, therefore further treatment is likely to be needed, albeit intermittently, throughout the individual's life. Apart from the large commitment this imposes on the child and his or her family, there must remain a degree of uncertainty as to the long-term effects of what is a relatively untested treatment. For this reason, treatment of childhood AA with DPC should be reserved for severe, long-standing disease, and should only be prescribed after the parents have been made completely aware of all possible side-effects to the treatment. At the same time it is worthy of note that many parents in this position are prepared to accept a degree of risk if there is any hope of restoring their child to 'normality'.

References

1 Monk B. Induction of hair growth in alopecia totalis with diphencyprone sensitisation. *Clin Exp Dermatol* 1989; 14: 154.

2 MacDonald Hull SP, Cunliffe WJ. Successful treatment of alopecia areata using the contact allergen diphencyprone. *Br J Dermatol* 1991; 124: 212–3.

3 Happle R, Hansen BM, Wiesner-Menzel L. Diphencyprone in the treatment of alopecia areata. *Acta Derm Venereol (Stockh)* 1983; 63: 49–52.

4 Wilkerson MG, Connor TH, Henkin J *et al.* Assessment of diphenylcyclopropenone for photochemically induced mutagenicity in the Ames assay. *J Am Acad Dermatol* 1987; 17: 606–11.

5 Wilkerson MG, Henkin J, Wilkin K. Diphenylcyclopropenone: Examination for potential contaminants, mechanisms of sensitisation, and photochemical stability. *J Am Acad Dermatol* 1984; 11: 802–7.

6 MacDonald Hull S, Cunliffe WJ, Norris JF. Alopecia areata treated with diphencyprone: is an allergic response necessary? *Br J Dermatol* 1990; 122: 716.

7 MacDonald Hull S, Norris JF. Diphencyprone in the treatment of long-standing alopecia areata. *Br J Dermatol* 1988; 119: 367–74.

British Journal of Dermatology (1991) **125**, 169–171. ADONIS 0007096391001887

Dowling–Degos disease and Kitamura's reticulate acropigmentation: support for the concept of a single disease

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Accepted for publication 12 February 1991

Summary Dowling–Degos disease and Kitamura's reticulate acropigmentation are both rare disorders of pigmentation, which predominantly affect the flexures and the extremities, respectively. A small number of patients with features of both disorders have been described, suggesting that these clinical patterns may be different manifestations of the same disease. We present a further case to support this conclusion.

Case report

A 56-year-old Caucasian man had a 20-year history of a pigmented rash that affected the axillae and neck. This had a rapid onset and, according to the patient, developed a few weeks after a large blood transfusion, and had gradually got worse. The eruption was asymptomatic except for episodic axillary soreness and pruritus. These episodes were associated with a change in the morphology of the eruption to form erythematous papules or erosions. This appearance had previously suggested a diagnosis of Hailey–Hailey disease (benign familial pemphigus) but his family history was negative and two previous skin biopsies had shown no evidence of acantholysis. Examination revealed widespread pigmentation which was most prominent in the axillae and on the face and sides of the neck (Fig. 1), but extended over the whole trunk and to all parts of the upper limbs except the palms (Fig. 2). Individual lesions were stellate small macules, which coalesced into a reticulate pattern around the neck and in the axillae. Confluent erythematous papules were also present in both axillae, and some pitted scars around the mouth.

A skin biopsy demonstrated patchy epidermal thinning with hyperkeratosis, and focal oedema and neutrophil polymorph exocytosis suggestive of superficial infection. Digitate epidermal prolongations with pigmentation at the tips were prominent (Fig. 3), and similar epithelial prolongations extended from the infundibulum of a hair follicle. There was intense focal

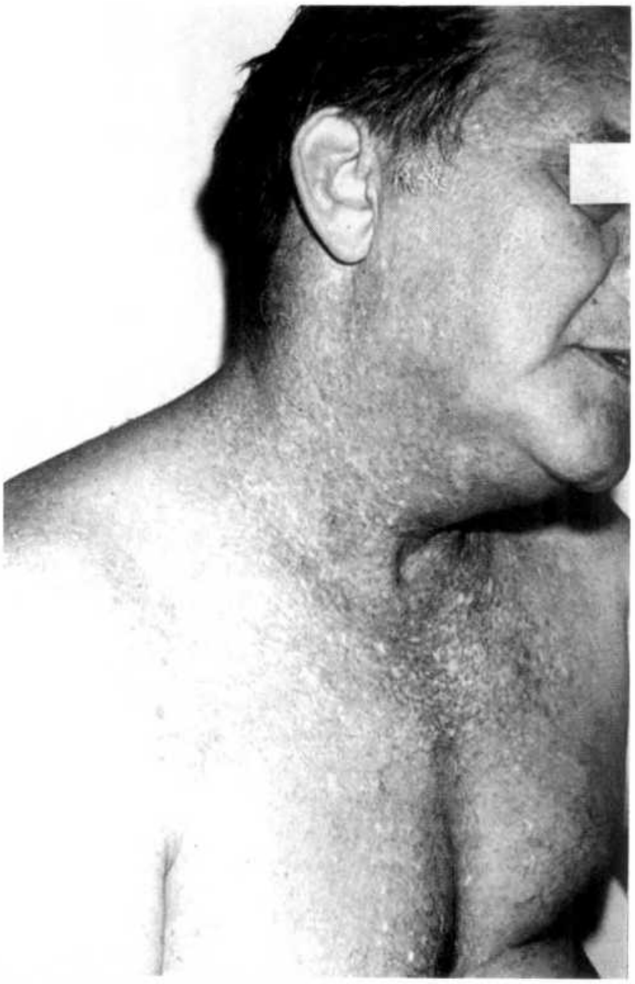

Figure 1. Reticulate pigmentation of the neck in Dowling–Degos disease.

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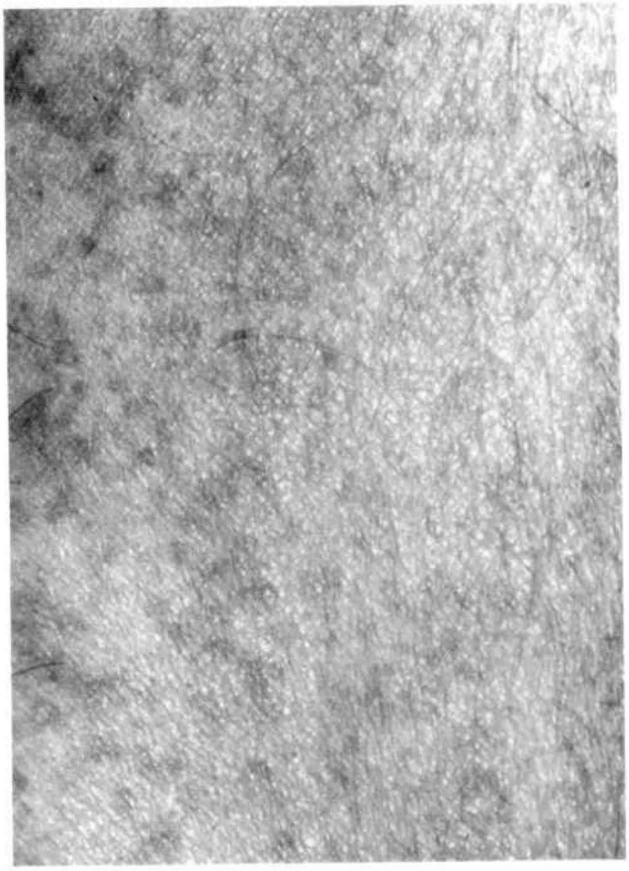

Figure 2. Pigmented macules on flexor aspect of wrist, suggestive of Kitamura's reticulate acropigmentation.

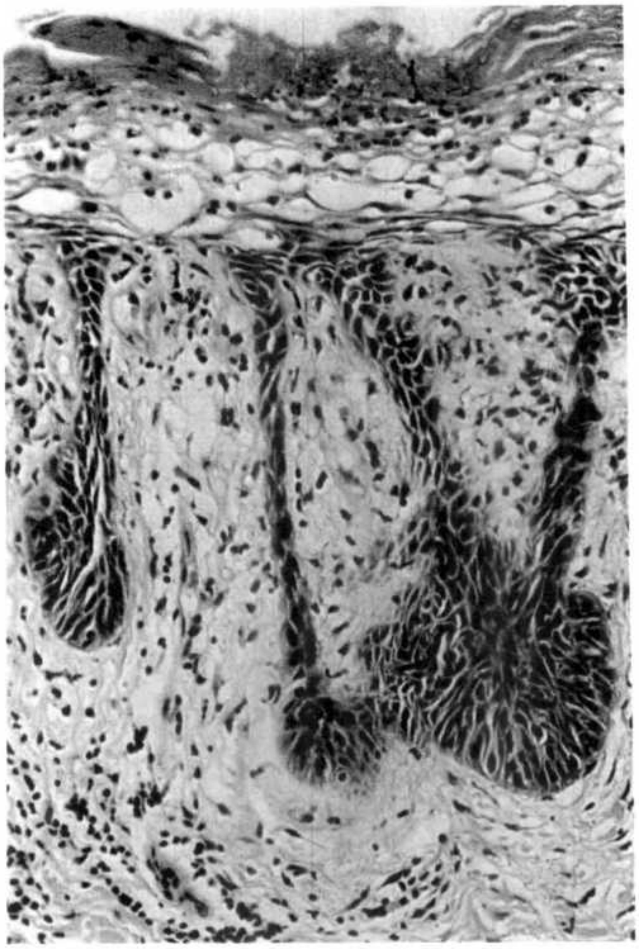

Figure 3. Histology of axillary skin biopsy. Digitate epidermal projections typical of Dowling–Degos disease, with superficial epidermal oedema due to secondary infection.

pigmentary incontinence. *Staphylococcus aureus* was cultured from the axillae, and the erythematous lesions responded to topical mupirocin ointment.

Discussion

The flexural pigmentation in this patient had the typical clinical and histological features of Dowling–Degos disease (DDD),[1–5] although the lack of other affected family members and the presence of secondary infection caused initial diagnostic difficulty. The focal pigmentary incontinence noted in the axillary biopsy may have been related to this episodic inflammation. A further unusual clinical feature was the presence of pigmented macules extending as far as the hands; these had the angulated freckle-like morphology of the lesions observed in two rare acral pigmentation disorders described by Kitamura and Akamatsu[6] and by Dohi,[7] although the lesions in our patient did not correspond absolutely with either of these disorders. In Kitamura's reticulate acropigmentation (KAR, acropigmentatio reticularis) the pigmented lesions are atrophic, a feature which was not clinically obvious in our patient. However, although the acral macules were not biopsied, there was mild epidermal atrophy in the axillary biopsy specimen. Palmar pits, which are usual but not invariable[8] in this disorder, were not present in our patient. The absence of hypopigmented macules excluded the diagnosis of reticulate acropigmentation of Dohi (dyschromatosis symmetrica hereditaria). The presence of KAR lesions in patients with DDD has been reported previously[4,9] and this was thought to be the most appropriate diagnosis in our patient. Several disorders have been brought into the spectrum of DDD by Rebora and Crovato,[2] following their description of a patient with features of both DDD and KAR.[4] It is interesting that their patient had a rapid onset of multiple extensive pigmented macules similar to that described by our patient. The family of their patient included cases of apparent DDD alone (mother) and KAR alone (sister), suggesting that these patterns of pigmentary abnormality are both part of the same disorder. A further family with more uniform features in the affected members has recently been described.[9] Rebora and Crovato reviewed other disorders which might also form part of this spectrum,[2] and concluded that a further six patients (five in one family,[10] and another originally diagnosed as xeroderma pigmentosum[11]) had been reported with coexistence of DDD and KAR, and that two patients diagnosed as cases of pigmentatio reticularis faciei and colli with epithelial cystomatosis had clinical features of DDD but the histology of KAR. Wilson Jones and Grice[1] had previously suggested that DDD has a wide spectrum; one of the previously reported cases discussed in their review of DDD had lesions extending to involve the wrists[12] and may therefore also have had the combination of DDD and KAR. Our patient adds support to the conclusion that such patients do not have chance coexistence of two very rare disorders, but that KAR is part of the spectrum of DDD.

References

1 Wilson Jones E, Grice K. Reticulate pigmented anomaly of the flexures: Dowling Degos disease, a new genodermatosis. *Arch Dermatol* 1978; **114:** 1150–7.

2 Rebora A, Crovato F. The spectrum of Dowling–Degos disease. *Br J Dermatol* 1984; **110:** 627–30.

3 Howell JB, Freeman RG. Reticular pigmented anomaly of the flexures. *Arch Dermatol* 1978; **114:** 400–3.

4 Crovato F, Desirello G, Rebora A. Is Dowling–Degos disease the same disease as Kitamura's reticulate acropigmentation? *Br J Dermatol* 1983; **109:** 105–10.

5 Griffiths WAD. Reticulate pigmentary disorders—a review. *Clin Exper Dermatol* 1984; **9:** 439–50.

6 Kitamura K, Akamatsu S. *Rinsho-no Hifu-Hitsunyo* 1943; **8:** 201. (cited by Griffiths[5]).

7 Dohi K. Dyschromatosis symmetrica hereditaria. Case presentation. *XVI International Congress of Dermatology*. (cited by Griffiths[5]).

8 Griffiths WAD. Reticulate acropigmentation of Kitamura. *Br J Dermatol* 1976; **95:** 437–43.

9 Berth-Jones J, Graham-Brown RAC. A family with Dowling–Degos disease showing features of Kitamura's reticulate acropigmentation. *Br J Dermatol* 1989; **120:** 463–6.

10 Becker SW, Reuter MJ. A familial pigmentary anomaly. *Arch Dermatol Syphilol* 1939; **44:** 987–98.

11 Montgomery H, Reuter MJ. Xeroderma pigmentosum. *Arch Dermatol Syphilol* 1932; **26:** 256–67.

12 Jacyk W, Miklaszewska M. Acquired axillary and inguinal pigmentations. *Dermatologica* 1973; **146:** 262–5.

British Journal of Dermatology (1991) **125**, 172–174. ADONIS 000709639100189K

Linear IgA disease with haemorrhagic pompholyx and dapsone-induced neutropenia

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Accepted for publication 7 March 1991

Summary A case of haemorrhagic pompholyx occurring in a 29-year-old man with linear IgA disease is described. There were several features in our patient that are usually seen in chronic bullous disease of childhood. Treatment with dapsone cleared the eruption but induced a progressive yet reversible neutropenia.

Case report

A 29-year-old man presented in October 1988 with a 4-month history of a blistering eruption which began on the face and spread to the upper back. He had chronic schizophrenia which was well controlled on sulpiride, but was not taking any other medication. Treatment was commenced with a moderately potent topical corticosteroid and oral flucloxacillin and this resulted in a dramatic response with complete clearing of all the skin lesions within 1 week. However, the rash relapsed 10 days later, and in addition to the previously affected sites, there was involvement of the groins, abdomen, mouth, scalp and palms. Examination revealed clear intact blisters, erosions and crust formation (Fig. 1); some areas in the groins also had an annular arrangement of blisters resembling a 'cluster of jewels'. There was severe mouth ulceration, and examination of the eyes by an ophthalmologist revealed a few superficial corneal erosions but no scarring. The palms showed hyperhidrosis and haemorrhagic blisters (Fig. 2) as well as sago-grain microvesicles. The feet were clear.

A biopsy of a representative lesion from the abdomen showed a subepidermal blister, papillary microabscesses and a predominence of eosinophils. Direct immunofluorescence on perilesional skin from the abdomen and palm demonstrated identical changes with a homogeneous and linear deposition of IgA along the basement membrane zone (BMZ). Indirect immunofluorescence revealed a circulating anti-BMZ IgA antibody at a titre of 1 : 32 using whole skin and a titre of 1 : 128 using saline-split skin. Standard haematological and biochemical parameters were normal, and there was no evidence of malabsorption. An endoscopic duodenal biopsy showed an increased number of chronic inflammatory cells in the lamina propria, but there was no evidence of villous atrophy.

In contrast to the marked improvement of the rash with a topical corticosteroid and a systemic antibiotic on the first occasion, this regimen failed to make an impression after the relapse. Treatment was thus changed to dapsone 100 mg daily, and increased to a dose of 150 mg daily after 1 week. This resulted in complete healing of the skin lesions and the mouth ulcers. However, dapsone produced a steady decline in the neutrophil count (Fig. 3), which continued for 1 week after stopping the drug. The lowest leucocyte count was $2{\cdot}2 \times 10^9$/l (neutrophils 22%; lymphocytes 64%; monoyctes 9% and basophils 5%) which was reached 6·5 weeks after starting dapsone. The haemoglobin level

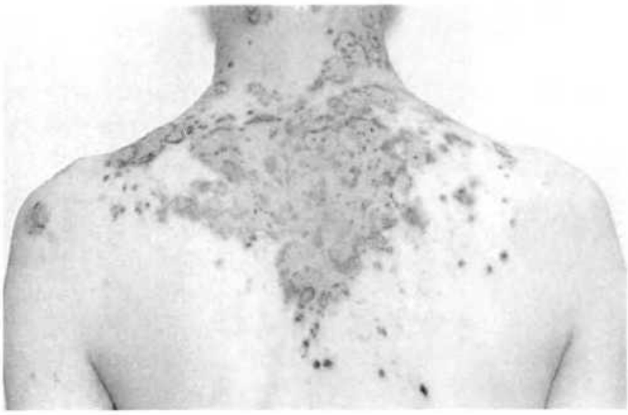

Figure 1. Blistering eruption with erosions on nape of neck and upper back.

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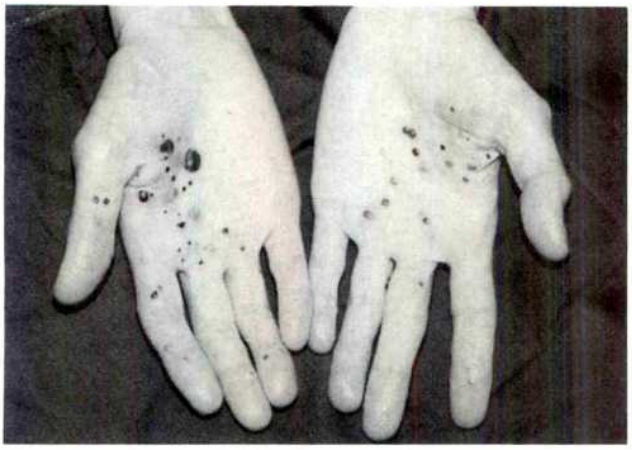

Figure 2. Haemorrhagic vesicles on the palms.

fell from a pretreatment value of 12·8 g/dl to 10·4 g/dl and the MCV rose from 83 to 99 fl. The platelet count remained normal. The patient did not develop any evidence of systemic infection during the short period of neutropenia. One year after the withdrawal of dapsone there has been no relapse of blisters or recurrence of neutropenia. Treatment with sulpiride has continued unaltered.

Discussion

Haemorrhagic pompholyx was described by Barth *et al.*[1] in three patients with bullous pemphigoid. It has also been reported in dermatitis herpetiformis[2,3] and has been observed in pemphigoid gestationis. The above case shows that it can also occur in adult linear IgA disease. Thus, haemorrhagic pompholyx can be a feature of a number of subepidermal blistering disorders, and it may not be as useful a sign of bullous pemphigoid as had been thought.[1] Haemorrhagic blisters have also been described occurring at sites distant from the palms and soles in three subepidermal blistering disorders. This suggests that haemorrhagic pompholyx simply represents a further manifestation of the underlying blistering disorder rather than a separate disease entity.

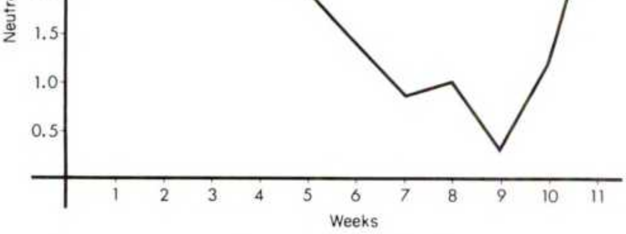

Figure 3. Fall in neutrophil count during treatment with dapsone, and recovery on withdrawal.

Adult linear IgA disease and chronic bullous disease of childhood (CBDC) used to be regarded as separate entities differing in the age of onset, extent of mucosal and perineal involvement, prognosis, circulating IgA anti-BMZ antibody titre and the incidence of HLA-B8.[4,5] However, there were several features of our case that are usually seen in CBDC, such as the extensive perineal and facial involvement, prolonged remission and the presence of circulating IgA anti-BMZ antibody. A considerable overlap in the clinical features between adult linear IgA disease and CBDC has recently been highlighted by Wojnarowska *et al.*[6] In addition, no absolute differences have been found between the two conditions and it has been suggested that they are the same disease.

Dapsone and sulphonamides are currently regarded as the treatment of choice for linear IgA disease[6] but both are liable to produce blood dyscrasias. Dapsone frequently induces a dose-dependent Coombs test negative haemolytic anaemia, particularly with doses above 100 mg daily. However, an adverse effect of dapsone on the neutrophil count is much rarer and is unpredictable. The precise mechanism of this effect is unclear but the generation of superoxide radicals and hydrogen peroxide by a metabolite of dapsone may be relevant in damaging the cell membrane of neutrophil precursors by peroxidation.[7] The factors determining the susceptibility of individuals to an adverse effect of dapsone on the neutrophil count also remain to be elucidated. The incidence of dapsone-induced agranulocytosis (a syndrome of neutropenia with systemic infection) has been reported to occur in 1 in 2000 to 1 in 5000 cases. This agranulocytosis becomes manifest 3–12 weeks after commencing dapsone and is associated with 50% mortality.[9] In most previous reports of dapsone-induced agranulocytosis the white cell count had not been checked after starting the drug until after the onset of a systemic infection.[8–11] Therefore it has not been clear whether the agranulocytosis develops abruptly or is preceded by a progressively worsening neutropenia. The above case demonstrates that dapsone can produce a progressive decline in neutrophil count and it is likely that agranulocytosis would have supervened if the drug had not been withdrawn. Thus, life-threatening agranulocytosis might be avoided by checking the full blood count after commencing dapsone, and stopping the drug if the neutrophil count shows a significant decrease.

Acknowledgment

We would like to thank the Department of Immunofluorescence, St John's Dermatology Centre (formerly St John's Hospital for Diseases of the Skin) for the indirect immunofluorescence findings.

References

1 Barth JH, Fairris GM, Wojnarowska F, White JE. Haemorrhagic pompholyx is a sign of bullous pemphigoid and an indication for low dose prednisolone therapy. *Clin Exp Dermatol* 1986; 11: 409–12.

2 Pierce DK, Purcell SM, Spielvogel RL. Purpuric papules and vesicles of the palms in dermatitis herpetiformis. *J Am Acad Dermatol* 1987; 16: 1274–6.

3 Massone L, Borghi S, Pestarino A *et al.* Dermatitis herpetiformis: A case with palmar purpuric lesions treated with disodium cromoglycate. *J Am Acad Dermatol* 1988; 19: 577.

4 Leonard JN, Haffenden GP, Ring NP *et al.* Linear IgA disease in adults. *Br J Dermatol* 1982; 107: 301–16.

5 Mobacken H, Kastrup W, Ljunghall K *et al.* Linear IgA dermatosis: a study of ten adult patients. *Acta Derm Venereol (Stockh)* 1983; 63: 123–8.

6 Wojnarowska F, Marsden RA, Bhogal B, Black MM. Chronic bullous disease of childhood, childhood cicatricial pemphigoid, and linear IgA disease of adults. *J Am Acad Dermatol* 1988; 19: 792–805.

7 Weetman RM, Boxer LA, Brown MP *et al. In vitro* inhibition of granulopoiesis by 4-amino-4'-hydroxylaminodiphenyl sulphone. *Br J Haematol* 1980; 45: 361–70.

8 Friman G, Nystrom-Rosander C, Jonsell G *et al.* Agranulocytosis associated with malaria prophylaxis with maloprim. *Br Med J* 1983; **286**: 1244–5.

9 Ognibene AL. Agranulocytosis due to dapsone. *Ann Intern Med* 1970; 72: 521–4.

10 Stickland JF, Hurdle ADF. Agranulocytosis probably due to dapsone in an infantry soldier. *Med J Aust* 1970; i: 959–60.

11 Smithurst BA, Robertson I, Naughton MA Dapsone-induced agranulocytosis complicated by gram-negative septicaemia. *Med J Aust* 1971; 1: 537–9.

British Journal of Dermatology (1991) **125**, 175–177.

ADONIS 000709639100190E

Bacillary angiomatosis associated with cytomegalovirus infection in a patient with AIDS

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Accepted for publication 27 February 1991

Summary A patient with AIDS is reported with the recently described bacillary angiomatosis and with cytomegalovirus infection (CMV).

Bacillary angiomatosis is a recently described bacterial complication occurring in patients with the acquired immunodeficiency syndrome (AIDS). It is caused by a Gram-negative bacillus that can be demonstrated in tissues with the Warthin–Starry stain and on electron microscopy. The micro-organism is similar to that which causes cat-scratch disease.[1–3] The first case was recognized retrospectively in 1983 by Stoler *et al.*[4] as an atypical subcutaneous infection in a patient with AIDS. Since then there have been 34 reported cases[5–9] in the literature. This distinct vascular disorder in patients with AIDS was described as epithelioid angiomatosis,[10] but in 1988 the term bacillary angiomatosis was proposed[6] pending an exact identification of the causative micro-organism.

We present a patient with AIDS with bacillary angiomatosis and CMV inclusions in the endothelial cells of the vascular lesions.

Case report

A 38-year-old Caucasian homosexual man with stage IV AIDS and with CMV choroido-retinitis and gastritis and candidal oesophagitis, presented with two nodular lesions on the right leg. No other lesions were present and these nodules slowly increased in size, the one on the right ankle later becoming ulcerated (Fig. 1).

The histology of the lesion on the right ankle showed vascular proliferations that extended from the reticular dermis to the subcutaneous tissue. These vascular structures varied in size and were set in an oedematous stroma that resembled granulation tissue. The vessels were lined with prominent endothelial cells and in the surrounding tissue there was an intense inflammatory infiltrate composed mainly of neutrophils with leucocytoclasis and abundant granular amphophilic material (Fig. 2). Electron microscopy showed numerous bacilli (Fig. 3). The Warthin–Starry stain failed to demonstrate the presence of bacilli. Intranuclear and intracytoplasmic inclusions, characteristic for CMV, were

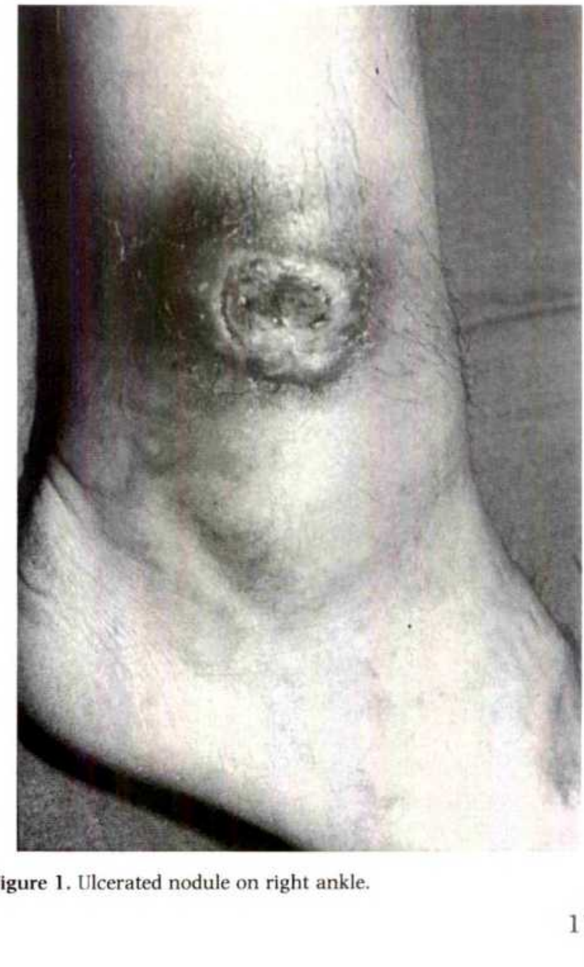

Figure 1. Ulcerated nodule on right ankle.

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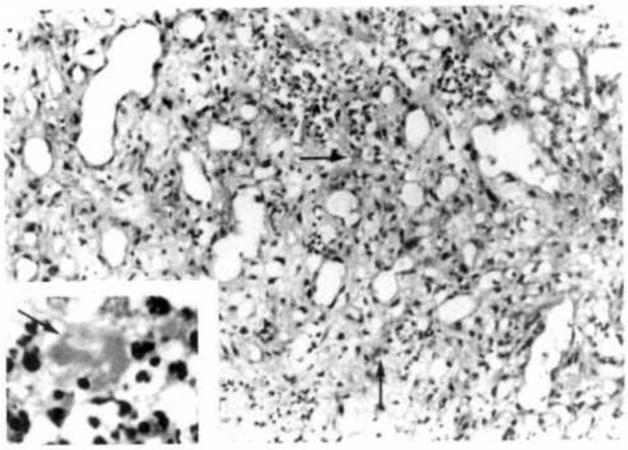

Figure 2. Vascular spaces lined by epithelioid endothelial cells. In the oedematous stroma there are numerous neutrophils as well as clumps of granular amphophilic materials (arrows) (haematoxylin and eosin, × 100). Inset: detail of the granular amphophilic material (arrow) (haematoxylin and eosin, × 400).

observed in some of the prominent endothelial cells in the vessels (Fig. 4). These inclusions were positive to an anti-CMV monoclonal antibody diluted to 1:25 and using an indirect immunoperoxidase method.

The patient was treated with oral isoniazid, rifampicin and pyrazinamide and after 2 months the lesions had completely resolved. However, the patient later died from the complications of disseminated CMV infection and at autopsy, in addition to the choroido-retinitis, there was evidence of CMV infection in the lung and gastrointestinal tract.

Discussion

Bacillary angiomatosis after Kaposi's sarcoma is the next most common angiomatous skin lesion to be associated with HIV-1 infection.[8] The histology is similar to that of pyogenic granuloma, although the endothelial cells are markedly atypical and adjacent to the vascular spaces there are numerous neutrophils with necrosis and granular material that contain clusters of bacilli. These bacilli have been considered to be identical or similar to those seen in cat-scratch disease. In our patient we had difficulty in identifying the bacilli using the Warthin–Starry stain, and we found that electron microscopy was of help in confirming the presence of these bacteria.[11]

The histopathology of our case resembled closely those previously described, although in our patient there were abundant inclusions indicative for CMV infection in the prominent endothelial cells. The presence of CMV was confirmed using the anti-CMV monoclonal antibody (CCH2), that is considered to be almost as specific as the *in-situ* hybridization technique.[12]

It has been reported that the *tat* gene of HIV induces dermal lesions similar to those of Kaposi's sarcoma in transgenic mice.[13] Also, pyogenic granulomas have been described in patients with terminal malignant disease,[14,15] in which there may be severe immunodepression not caused by HIV-1 virus. Also, recently a case has been described of bacillary angiomatosis in an immunocompetent patient.[9]

Cytomegalovirus DNA has been detected in both classical and epidemic Kaposi's sarcoma by *in-situ* hybridization[16] and the angiogenic capacity of this virus

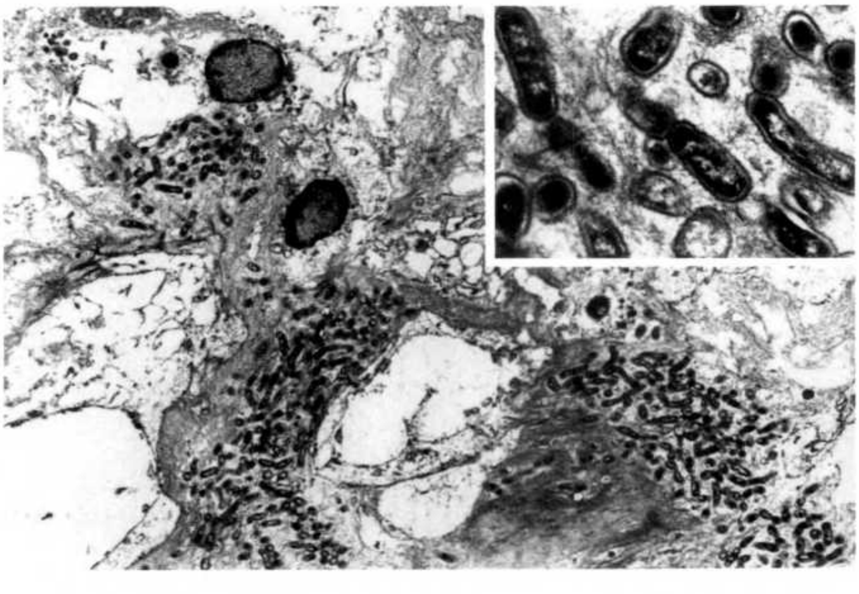

Figure 3. Electron micrograph showing numerous extracellular bacillary structures (original × 2200). Inset: higher magnification showing trilaminar wall structures typical for Gram-negative bacilli (original × 11,500).

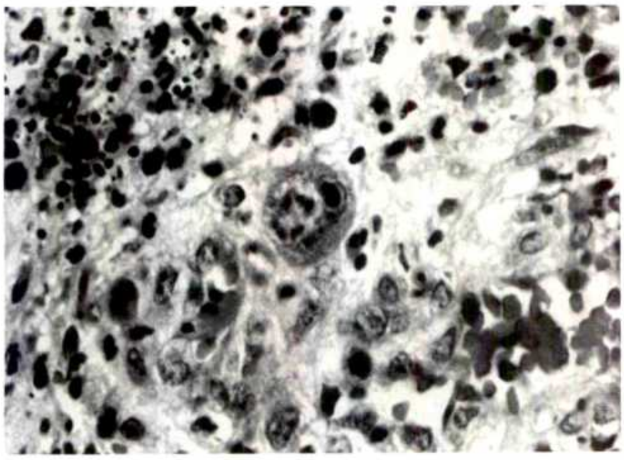

Figure 4. Intranuclear and intracytoplasmic inclusions diagnostic for cytomegalic infection (haematoxylin and eosin, × 400).

is known.[17] However, the association of CMV with bacillary angiomatosis is unusual and we have found only one previous case in the literature.[7] We believe that the CMV infection was not directly responsible for the development of the lesions of bacillary angiomatosis. The bacillus of cat-scratch disease is probably the causative agent and this could produce substances that have a direct effect in vessels and induce proliferation. However, there is little clinical and histological similarity between the condition of bacillary angiomatosis and cat-scratch disease. Also, only one case of cat-scratch disease in a patient with AIDS has been previously reported.[18] Although the DNA of the Epstein–Barr virus has recently been demonstrated in the endothelial cells in patients with bacillary angiomatosis with AIDS, the pathogenic role of this virus remains to be clarified.[19]

In conclusion, bacillary angiomatosis is an infectious disease that is almost exclusively seen in patients with HIV infection. Treatment of the condition with appropriate antibiotics is indicated.

References

1 Le Boit PE, Berger TG, Egbert BM *et al.* Epithelioid haemangioma-like vascular proliferation in AIDS: manifestation of cat scratch disease bacillus infection? *Lancet* 1988; **i:** 960–3.

2 English CK, Wear DJ, Margileth AM *et al.* Cat-scratch disease: isolation and culture of the bacterial agent. *JAMA* 1988; **259:** 1347–52.

3 Le Boit PE. The expanding spectrum of a new disease, bacillary angiomatosis. *Arch Dermatol* 1990; **126:** 808–11.

4 Stoler MH, Bonfiglio TA, Steigbigel RT, Pereira A. An atypical subcutaneous infection associated with acquired immune deficiency syndrome. *Am J Clin Pathol* 1983; **80:** 714–18.

5 Jimenez-Acosta F, Pardo RJ, Cohen RJ *et al.* Bacillary angiomatosis of acquired immunodeficiency syndrome. Case report and literature review. *J Am Acad Dermatol* 1990; **22:** 525–9.

6 Le Boit PE, Berger TG, Egbert BM *et al.* Bacillary angiomatosis. The histopathology and differential diagnosis of a pseudoneoplastic infection in patients with human immunodeficiency virus disease. *Am J Surg Pathol* 1989; **13:** 909–20.

7 Abrams J, Farhood AI. Infection-associated vascular lesions in acquired immunodeficiency syndrome patients. *Hum Pathol* 1989; **20:** 1025–6.

8 Berger TG, Tappero WJ, Kaymen A, Le Boit PE. Bacillary (epithelioid) angiomatosis and concurrent Kaposi's sarcoma in acquired immunodeficiency syndrome. *Arch Dermatol* 1989; **125:** 1543–7.

9 Cockerell CJ, Bergstresser PR, Myrie-Williams C, Tierno PM. Bacillary epithelioid angiomatosis occurring in an immunocompetent individual. *Arch Dermatol* 1990; **126:** 787–90.

10 Cockerell CJ, Whitlow MA, Webster GF, Friedman-Kien AE. Epithelioid angiomatosis: a distinct vascular disorder in patients with the acquired immunodeficiency syndrome or AIDS related complex. *Lancet* 1987; **ii:** 654–6.

11 Cockerell CJ, Le Boit PE. Bacillary angiomatosis: a newly characterized pseudoneoplastic, infectious, cutaneous vascular disorder. *J Am Acad Dermatol* 1990; **22:** 501–12.

12 Niedobitek G, Finn T, Herbst H *et al.* Detection of cytomegalovirus by *in situ* hybridization and immunohistochemistry using new monoclonal antibody CCH2: a comparison of methods. *J Clin Pathol* 1988; **41:** 1005–9.

13 Vogel J, Hinrichs SH, Reynolds RK *et al.* The HIV Tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* 1988; **335:** 606–11.

14 Omura EF, Omura GA. Human immunodeficiency virus-associated skin lesions. *JAMA* 1989; **261:** 991.

15 Nappi O, Wick NR. Disseminated lobular capillary hemangioma (pyogenic granuloma: a clinico-pathologic study of two cases). *Am J Dermpathol* 1986; **8:** 379–85.

16 Grody WW, Lewin KJ, Naeim F. Detection of cytomegalovirus DNA in classic and epidemic Kaposi's sarcoma by *in situ* hybridization. *Hum Pathol* 1988; **19:** 524–8.

17 Langford A, Kunre R, Timm H *et al.* Cytomegalovirus associated oral ulcerations in HIV infected patients. *J Oral Pathol Med* 1990; **19:** 71–6.

18 Pilon VA, Echols RM. Cat-scratch disease in a patient with AIDS. *Am J Clin Pathol* 1989; **92:** 236–40.

19 Guarner J, Unger ER. Association of Epstein–Barr virus in epithelioid angiomatosis of AIDS patients. *V International Conference on AIDS*. Montreal June 1989, Abstract No. M.B.P. 62, p. 232.

British Journal of Dermatology (1991) **125**, 178–182. ADONIS 000709639100191R

Precocious puberty in a boy with a widespread linear epidermal naevus

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Accepted for publication 27 February 1991

Summary A 7-year-old boy with a systematized verrucous epidermal naevus, multiple pigmented naevocytic naevi and precocious puberty is described. The possible basis for this previously unreported association of abnormalities is discussed.

A linear epidermal naevus may be accompanied by other abnormalities. The term epidermal naevus syndrome[1] refers to its association with neurological, ocular, skeletal or other abnormalities. We describe a child with a systematized epidermal naevus and precocious puberty, an association not previously described.

Case report

A male infant, M.W., birth weight 4·0 kg, initially presented aged 6 weeks with a urinary tract infection. Hepatomegaly was noted at 2 months, enlarged genitalia at 18 months, and pubic hair and accelerated growth by 2 years. Numerous brown streaks on the skin were first observed at 2 years and have become more prominent. Between the ages of 3 and 4 years, bilateral inguinal hernias were repaired. He attends a normal school but requires remedial reading lessons. For the past year he has suffered occasional absence attacks.

He is the only child of healthy, non-consanguineous parents. His mother had been treated for infertility, but was on no medication when she conceived or during pregnancy. She had had 12 siblings, four of whom had serious diseases, namely fatal heart disease in infancy, a bleeding tendency, hydronephrosis requiring nephrectomy at age 16, and fatal malignant hyperpyrexia. This last diagnosis was excluded in the mother on muscle biopsy.

Physical examination

On examination, aged 8 years, his height (141 cm) and weight (41 kg) were well above the 97th centile. He was somewhat brachycephalic and prognathic (Fig. 1). His whole body was affected by brown streaks and whorls, warty on the neck and just palpable on the trunk and limbs, distributed in Blaschko's lines (Figs 2 and 3). Scalp hair arising from abnormal skin was short, coarse, blond and wavy, compared with the normal scalp terminal hairs. The abnormal hairs were shed more often, suggesting a short cycle time. Eight weeks after cutting,

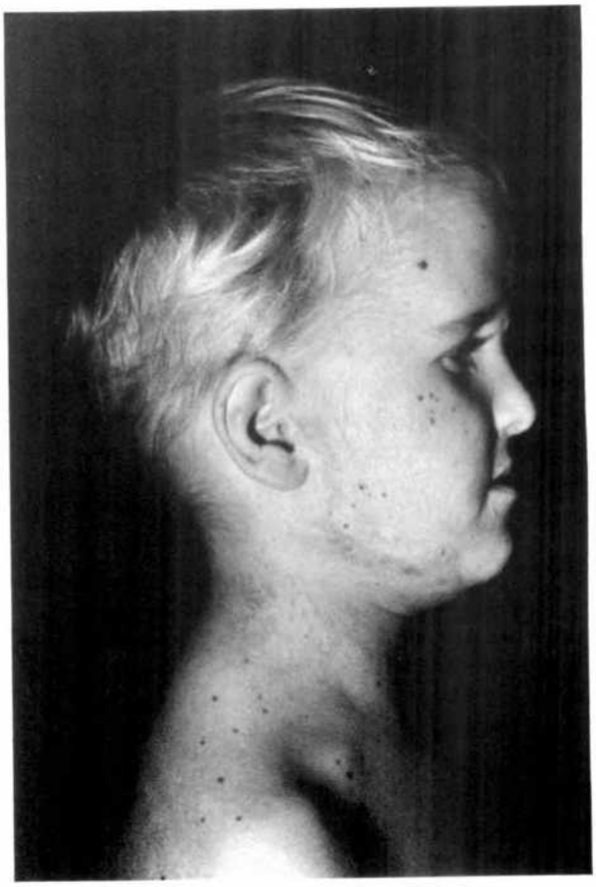

Figure 1. Brachycephaly and prognathism plus multiple naevi.

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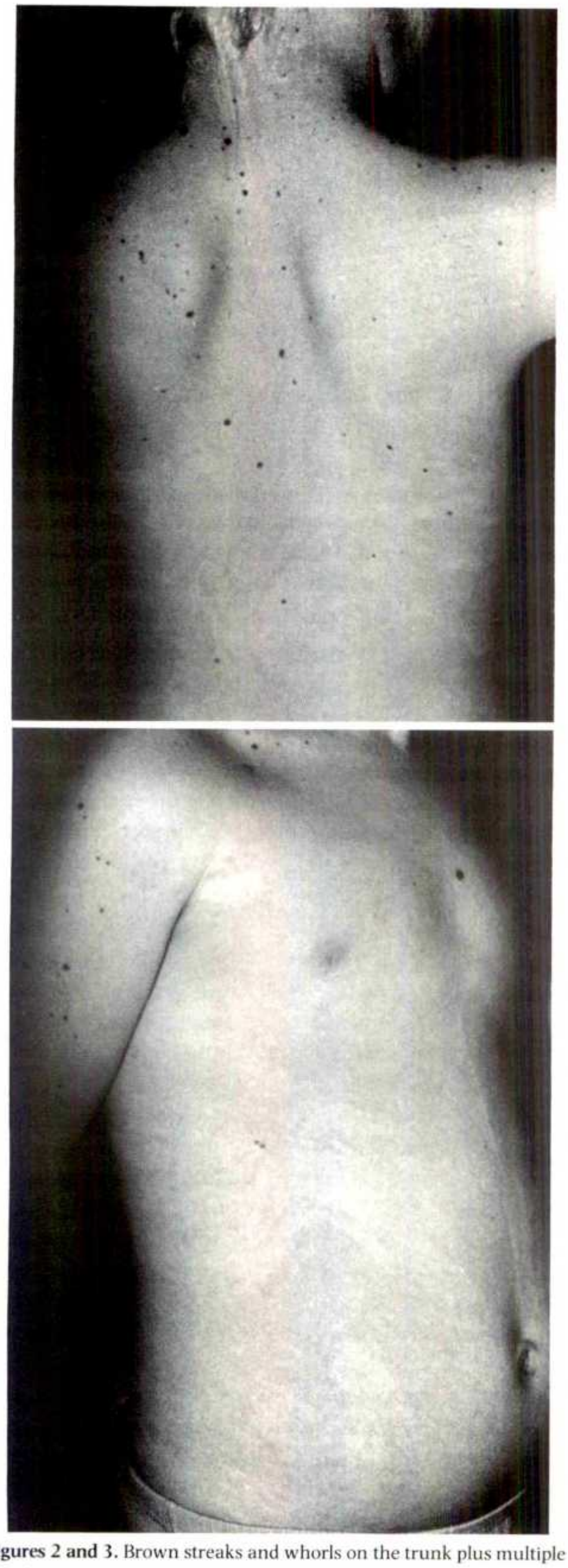

Figures 2 and 3. Brown streaks and whorls on the trunk plus multiple naevi.

the abnormal hairs had grown 1 cm less than adjacent normal hairs, so the rate of growth was also reduced. On the trunk and limbs hair growth on the abnormal skin was greater than that of adjacent vellus hair. There were multiple darkly pigmented raised naevi up to 7 mm in diameter distributed asymmetrically mainly on the head and upper trunk, occurring on both normal and verrucous skin (Figs 1 and 2). His nails and teeth were normal apart from dental malocclusion and advanced dental maturity. He had acne, a deep voice, axillary and pubic hair, enlarged testes (15 c.c.), and a proportionately enlarged penis. The liver, which in infancy had been smoothly enlarged to 2 cm below the coastal margin, was no longer palpable. Ocular and neurological examinations were normal apart from poor hand–eye co-ordination. Psychological examination revealed normal overall intelligence, with a reading disability due to a specific problem with perception of shapes.

Investigations

At 6 weeks, two mid-stream urine specimens contained significant numbers of pus cells and *Escherichia coli*. Blood urea and electrolyte levels were normal, apart from a low bicarbonate of 11 mmol/l (normal 25–32 mmol/l) with a urinary pH of 6·7 indicating renal tubular acidosis. An intravenous pyelogram showed left pelvicalyceal dilatation. An ^{123}I scan showed normal concentration in both kidneys, but no drainage from the left kidney until a diuretic was administered, suggesting dilatation without obstruction. A recent renal ultrasound examination showed the left kidney to be smaller with slight calyceal dilatation and parenchymal loss at the upper pole, and renal function is now normal.

Investigation of the hepatomegaly revealed normal liver function tests, glucose, and α-foeto protein, but α-1 antitrypsin was reduced at 1·3 g/l (normal range 1·8–3·0) with a heterozygous (PiMS) phenotype. Both parents were also heterozygous (PiMS) with α-1 antitrypsin levels of 1·0 g/l (mother) and 1·5 g/l (father). Urinary screening for elevated amino acids, organic acids and mucopolysaccharides was negative. A skeletal survey at 2 months revealed bilateral coxa vara and deformity of the proximal ends of both radii. There was no fibrous dysplasia. The bone age was 4·7 years at chronological age 2·1 years, rising to 11·7 at 5 years and 15·7 at 6·8 years.

Skin biopsies were taken for routine histology, electron microscopy and fibroblast culture. Lesional skin on the trunk compared with adjacent normal skin showed only the acanthosis, papillomatosis and hyperkeratosis characteristic of a verrucous epidermal naevus and these changes were more prominent in the more warty lesional skin of the neck. There was no inflammation or epidermolytic change. A dark mole from the chest was histologically a pigmented compound naevocytic naevus. Fibroblasts cultured from both affected and unaffected skin showed normal 46XY karyotype with no chromosomal abnormalities or mosaicism. Melanocytes appeared normal on electron microscopy.

Full endocrine evaluation showed only the following abnormalities. Testosterone level (nmol/l) was 1·2 at 3 years 1 month, 10·8 at 4 years, 12·0 at 6 years and less than 1·0 at 8 years (normal adult male values 8·5–28·4 nmol/l). An LHRH stimulation test at 2 years gave the following results at times 0, 30 and 60 min, respectively (with normal unstimulated adult male values in brackets): LH 6·3, 31, 27 U/l (1–6 U/l); FSH 1·7, 2·4, 2·9 U/l (1–7 U/l). An overnight gonadotrophin profile at age 6 years showed characteristic pubertal LH pulses, which were subsequently suppressed by Decapeptyl. Basal and stimulated levels of cortisol and growth hormone were normal. Skull X-ray and electroencephalogram were normal, as was cranial computerized tomography on four occasions. Magnetic resonance imaging is awaited.

Management

Treatment with cyproterone acetate 50–100 mg daily from age 2 years 4 months to 5 years did not slow his development. The long-acting LHRH analogue buserilin 200 μg was administered three times daily intranasally for the following year, but was poorly tolerated because of rhinitis and was ineffective. However, monthly intramuscular injections of a long-acting LHRH analogue, Decapeptyl, at a dose of 2·75 mg (66 μg/kg), commenced at age 6 years, have improved his acne and reduced the frequency of penile erections. His growth has slowed over the last 2 years, probably because of epiphyseal fusion.

Discussion

The major cutaneous abnormality in this patient is verrucous epidermal naevus. The relatively smooth texture, light brown colour and wide distribution of this patient's lesions are unusual, but have been described in other cases.[2] The terminology of epidermal naevi is confused. Some authors would use the term ichthyosis hystrix for such an extensive bilateral epidermal naevus, but most would confine this term to a linear epidermal naevus with the histological features of epidermolytic hyperkeratosis. The lesions biopsied in this patient showed only verrucous epidermal naevus and not sebaceous naevus, comedone naevus or ichthyosis hystrix, but these histological variants may be found in the same patient at different times and at different sites, even within the same lesion.[3] Therefore Solomon and Esterley regarded them all as variants of the same abnormality and grouped them all under the title 'epidermal naevus'.[2] Inflammatory linear verrucous epidermal naevus is probably a distinct entity.[4]

The pattern of Blaschko's lines is well shown in this patient, with lesions radiating laterally round the trunk from a V at the back and meeting in the midline anteriorly, whorling on the flanks, and extending linearly along the limbs. The lines radiate laterally from the corners of the mouth and appear to spiral around the scalp. The direction of Blaschko's lines is most readily explained by the clonal expansion and migration of ectodermal cells outwards from the dorsal primitive streak.[5,6]

Sexual precocity in this boy was due to premature maturation of the hypothalamic–pituitary axis ('true' precocious puberty), rather than autonomous end-organ activity ('pseudopuberty'). No cause has been found but a tumour must be suspected. Precocious puberty is four to five times more common and more often idiopathic in girls, a hormone-secreting tumour being identifiable in 80–90% of boys.[7] Because this boy had hepatomegaly, hepatoblastoma with ectopic gonadotrophin production was considered. The gonadotrophin may be HCG which cross-reacts with LH in immunoassay.[8] This diagnosis has however been excluded by a normal serum α-foeto protein level, and by the exaggerated LH response to LHRH which is diagnostic of precocious puberty of central origin. In some children previously thought to have idiopathic precocious puberty, small hypothalamic hamartomas have been found. These are isodense tumours which may not be seen on computerized tomography but only on magnetic resonance imaging.[9] A small benign LH-secreting hypothalamic hamartoma remains a possibility in this patient.

Sexual precocity due to McCune–Albright syndrome was excluded in our patient firstly by the absence of polyostotic fibrous dysplasia on skeletal survey, and secondly by the absence of the typical flat, non-verrucous asymmetric pigmented patches which do not usually conform to Blaschko's lines, although Happle has argued that they may do so.[10] Finally precocious puberty in McCune–Albright syndrome is usually gonadotrophin-independent and responds to treatment with cyproterone, a peripherally acting agent, rather than LHRH agonists, which work centrally by suppressing the pulsatile release of gonadotrophin necessary for true puberty. In some patients with McCune–Albright syndrome the early elevation of sex steroids due to pseudopuberty may initiate the early maturation of the hypothalamic–pituitary axis and premature true puberty,[11] but in this boy gonadotrophins were elevated from the age of 2 years. Interestingly, epidermal naevi have been reported in three patients with polyostotic fibrous dysplasia.[12–14] All were male, none had precocious puberty, and in all the predominant cutaneous abnormality was widespread verrucous epidermal naevus mainly on the upper part of the body.

Sexual precocity and pigmented spots occur together in the Carney complex,[15] but this diagnosis can be dismissed for the following reasons. Firstly, the various endocrine disturbances reported in the Carney complex are due to end-organ dysfunction, adrenocortical disease being the most frequent, and precocious puberty arising from testicular tumours. In this patient the only endocrine abnormality was precocious puberty and this was of central origin. Secondly, the cutaneous features of the Carney complex, namely lentigines, blue naevi and myxomas in a predominantly mid-facial distribution, are quite different from those in our patient who had compound melanocytic naevi and epidermal naevi widely distributed on the head and trunk. Thirdly, there was no clinical evidence of atrial myxoma. Particular attention was paid to the cardiovascular system in our patient because his radial abnormality resembled that seen in Holt–Oram syndrome in which cardiac anomalies occur and which has been described in association with α-1-antitrypsin deficiency.[16] Echocardiography was not indicated.

Several of this patient's abnormalities have previously been reported in association with linear epidermal naevi. His bony abnormalities include the unusual shape of the cranium, a large jaw and deformities of the radial heads, in addition to changes secondary to precocious puberty. The skeletal changes found in other patients were extremely varied but included hypertrophy of cranial bones, and hypoplasia of limb bones. Inguinal hernias occurred in this patient and in five of 119 patients with epidermal naevi[17] but this may be a chance association. Renal abnormalities in previous patients included horseshoe kidney, ectopic ureter, double collecting system, vitamin D-resistant rickets and nephroblastoma, while our patient had pelvicalyceal dilatation with infection and transient renal tubular acidosis. He also has multiple compound pigmented naevocytic naevi. Their rapid proliferation may be partly due to his precocious puberty. Multiple pigmented naevi were found in six out of 60 patients with the epidermal naevus syndrome,[2] and in a patient reported by Sugarman and Reed.[18] One of the patients with epidermal naevus and polyostotic fibrous dysplasia[13] appears from the illustrations to have had numerous melanocytic naevi as well as a coloboma of the eyelid. The pigmented naevi are not localized to the linear epidermal naevus. The neurological abnormalities most commonly associated with epidermal naevi are mental retardation and epilepsy. This patient has recently developed absence attacks suggestive of petit mal epilepsy, with a normal electroencephalogram between attacks. He has a specific learning disability, which, had it not been identified and treated early, would have led to profound educational delay. Endocrinopathy is not associated with epidermal naevi except in one adult male who developed a pituitary tumour, presenting with headache, hypogonadism and hypothyroidism.[19] One child with an epidermal naevus developed the diencephalic syndrome of emaciation and was found to have a hypothalamic hamartoma but had no apparent endocrine abnormalities.[20]

The association of a linear epidermal naevus of either verrucous or sebaceous type with abnormalities in other systems, particularly the nervous system, eye and skeleton[1,21] has been called the epidermal naevus syndrome.[1] The syndrome has never been defined precisely and the associated abnormalities are extremely variable. The estimated frequency of the various associated abnormalities is subject to ascertainment bias. Thus in a series of 37 patients reviewed in the neurological literature,[22] 100% had neurological involvement, and the incidence of skeletal and ocular abnormalities was 65 and 46%, respectively. In a recent study of 119 patients with epidermal naevus presenting to dermatologists, 16% had two or more other abnormalities: 15% had neurological abnormalities, 15% skeletal and 9·6% ocular.[17] Solomon and Esterly reported 60 patients with the epidermal naevus syndrome of whom 50% had severe to moderate neurological impairment and 33% had ocular manifestations.[2] They did not state their diagnostic criteria, but it is clear from the figures that they did not regard either ocular or neurological abnormalities as essential. Patients with epidermal naevi and localized gigantism have been regarded as a distinct entity but even this subgroup is so variable that it has been called Proteus syndrome[23] after the Greek god who could change his form.

It seems likely that the diverse abnormalities in this syndrome are linked by a common pathogenesis, namely a failure in tissue organization including a particular tendency to form hamartomas, ie. benign tumours present at or soon after birth and containing normal tissues in abnormal proportions. Verrucous epidermal naevi can themselves be considered hamartomas and are commonly associated with other hamartomas, in particular haemangiomas and melanocytic naevi in the skin[15] and vascular or neural tumours in the brain.[22,24] This patient has two types of cutaneous hamartoma, epidermal and melanocytic, and he may also have a hypothalamic hamartoma. Other neurocutaneous syndromes, or phakomatoses, such as tuberous sclerosis and von Recklinghausen's neurofibromatosis also comprise hamartomas in various organs, but the range of tumours found is more limited than in the epidermal naevus syndrome.

The cause of epidermal naevi is unknown. The parents of our patient share the same rare protease inhibitor phenotype, PIMS, carried by less than 5% of the population, suggesting that they may unknowingly be consanguineous and that their son's disorder may be recessively inherited. However in general patients with epidermal naevi have no family history of either the skin disorder or consanguinity, and the disorder is more likely to reflect a new mutation. If Blaschko's lines are indeed the boundaries between different clones of cells this cutaneous mosaic pattern may represent genetic mosaicism. We suggest that the linear skin lesions in this patient represent clones of cells carrying a somatic mutation too small to be detected by cytogenetic analysis.

The spatial distribution of the neurological lesions is also of interest. In almost all patients with linear epidermal naevi and neurological abnormalities, the naevus on the head is ipsilateral to the brain pathology:[25] it may be that an ectodermal abnormality induces abnormal development in the underlying mesoderm.[25] Perhaps a comparison of genetic material from the lesional and non-lesional skin will reveal the basis of this patient's disorder.

Acknowledgment

We are grateful to Professor A.Aynsley-Green for helpful comments.

References

1 Solomon LM, Fretzin DF, Dewald RL. The epidermal nevus syndrome. *Arch Dermatol* 1968; **97**: 273–85.

2 Solomon LM, Esterly NB. Epidermal and other congenital organoid nevi. In: *Current Problems in Pediatrics* Vol VI, no. 1. Chicago: Medical Year Book Publishers, 1975; 2–56.

3 Mehregan AH, Pinkus H. Life history of organoid nevi. *Arch Dermatol* 1965; **91**: 574–88.

4 Moss C, Burn J. ILVEN+CHILD=PEN or PENCIL. *J Med Genet* 1990; **27**: 390–1.

5 Shuster S. *Dermatology in Internal Medicine.* Oxford: Oxford University Press, 1978; 21.

6 Happle R. Lyonisation and the lines of Blaschko. *Hum Genet* 1985; **70**: 200–6.

7 Editorial. Precocious puberty. *Lancet* 1986; **ii**: 80–2.

8 Navarro C, Corretger JM, Sancho A *et al.* Paraneoplastic precocious puberty: report of a new case with hepatoblastoma and review of the literature. *Cancer* 1985; **56**: 1725–9.

9 Hahn FJ, Leibrock LG, Huseman CA, Makos MM. The magnetic resonance appearance of hypothalamic hamartoma. *Neuroradiol* 1988; **30**: 65–8.

10 Happle R. The McCune–Albright syndrome: a lethal gene surviving by mosaicism. *Clin Genet* 1986; **29**: 321–4.

11 Kaufman FR, Costin G, Reid BS. Autonomous ovarian hyperfunction followed by gonadotrophin-dependent puberty in McCune–Albright syndrome. *Clin Endocrinol* 1986; **24**: 239–42.

12 Grun G, Didier MF. Le syndrome d'Albright. A propos de deux observations. *Bull Soc Franc Derm Syph* 1972; **79**: 184–5.

13 Pierini AM, Ortonne JP, Floret D. Signes dermatologiques du syndrome de McCune–Albright: a propos d'un cas *Ann Dermatol Venereol* 1981; **108**: 969–76.

14 Rustin MHA, Bunker CB, Gilkes JJH *et al.* Polyostotic fibrous dysplasia associated with extensive linear epidermal naevi. *Clin Exp Dermatol* 1989; **14**: 371–5.

15 Carney JA, Gordon H, Carpenter PC *et al.* The complex of myxomas, spotty pigmentation and endocrine overactivity. *Medicine* 1985; **64**: 270–83.

16 Turleau C, de Grouchy J, Chavin-Colin F *et al.* Two patients with interstitial del (14q) one with features of Holt–Oram syndrome. Exclusion mapping of PI (alpha-1-antitrypsin). *Ann Genet* 1984; **27**: 237–40.

17 Rogers M, McCrossin I, Commens C. Epidermal nevi and the epidermal nevus syndrome. *J Am Acad Dermatol* 1989; **20**: 476–88.

18 Sugarman GI, Reed WB. Two unusual neurocutaneous disorders with facial cutaneous signs. *Arch Neurol* 1969; **21**: 242–7.

19 Abou Zeid SA, Khalil SA, Meheesen AM *et al.* Epidermal nevus with cutaneous endocrinal associations. *Arch Dermatol* 1979; **115**: 625–6.

20 Meyerson LB. Nevus unius lateralis, brain tumour, and diencephalic syndrome. *Arch Dermatol* 1967; **95**: 501–4.

21 Feuerstein RC, Mims LC. Linear nevus sebaceous with convulsions and mental retardation. *Am J Dis Child* 1962; **104**: 675–9.

22 Zaremba J. Jadassohn's naevus phakomatosis: 2 A study based on a review of thirty-seven cases. *J Ment Defic Res* 1978; **22**: 103–23

23 Wiedemann H-R, Burgio GR, Aldenhoff P *et al.* The proteus syndrome. *Eur J Pediatr* 1983; **140**: 5–12

24 Lansky LL, Funderburk S, Cuppage FE *et al.* Linear sebaceous nevus syndrome. a hamartoma variant. *Am J Dis Child* 1972; **123**: 587–90.

25 Baker RS, Ross PA, Baumann RJ. Neurologic complications of the epidermal nevus syndrome. *Arch Neurol* 1987; **44**: 227–32.

British Journal of Dermatology (1991) **125**, 183–185.

ADONIS 0007096391001926

Recurrent facial oedema associated with a silicone-rubber implant

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Accepted for publication 13 February 1991

Summary A 40-year-old man presented with facial oedema that resulted from an inflammatory reaction to a silicone-rubber implant inserted 16 years previously for an augmentation rhinoplasty.

Case report

A 40-year-old man presented with a history of intermittent periorbital oedema over the preceding 3 years. At the onset he had had a severe upper respiratory tract infection and had been treated with systemic antibiotics. Initially both eyes became intermittently uncomfortable, but gradually there was a worsening of his periorbital oedema which was always more marked on wakening. The condition was specifically aggravated by stooping or entering a hot atmosphere and he was also aware that the flushing became more severe after hot drinks, alcoholic beverages and spicy foods. He had received systemic antihistamine treatment without benefit and both specialist ophthalmological and ENT examinations were normal.

He gave a history of acne vulgaris as a teenager. During childhood, he had injured his nose at football and at the age of 16 years had undergone a submucous resection of part of the nasal septum, followed 5 months later by an augmentation rhinoplasty using a silicone-rubber implant to correct bridge-line depression.

On examination he had obvious oedema of his face most prominently in his cheeks and forehead. There was telangiectasia and evidence of previous acne scarring. There was no lymphadenopathy (Fig. 1).

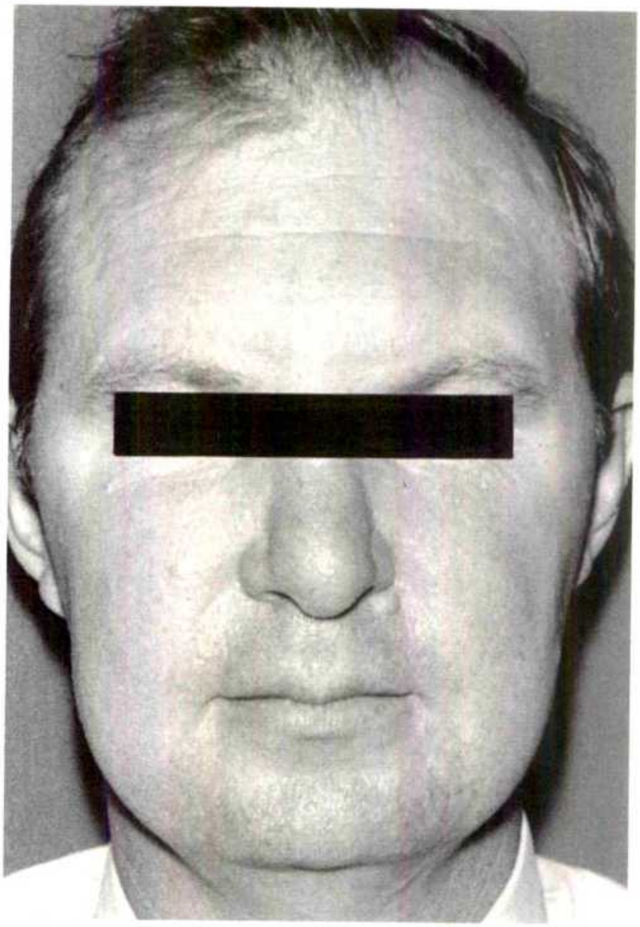
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Figure 1. Oedema of the face.

Investigations

The following investigations were normal: full blood count, ESR, ASO titre, serum IgE and RAST; chest X-ray, sinus X-ray, and CT scan of skull; antinuclear factor; creatinine kinase; *Filaria* complement fixation test; *Trichinella* CIE; 99 mTc, HM-PAO labelled white-cell scan of nasal passages or paranasal sinuses; and urinary porphyrins and 5-hydroxyindoleacetic acid.

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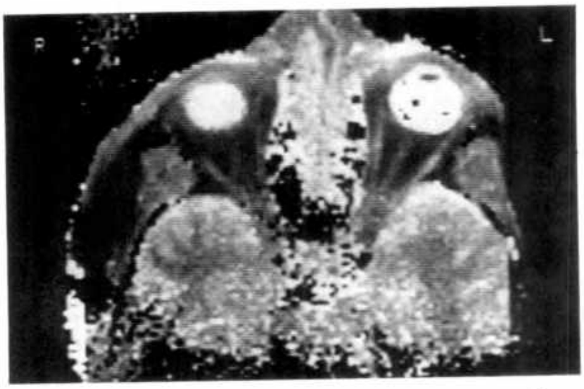

Figure 2. Transverse calculated T1 magnetic resonance image through both orbits demonstrating bilateral periorbital oedema (white) lying in front of the eyes in the medial aspect of the eyelids and marked anterior nasal mucosal thickening and oedema. No ocular or orbital abnormality is seen.

A skin biopsy of the cheek revealed alcianophilic solar elastosis, a perivascular chronic inflammatory infiltrate and upper dermal oedema. A nasal swab was positive for *Staphylococcus aureus*. Nuclear magnetic resonance imaging was performed and the images were made using an 0.08 Tesla, resistive MR imager designed and built at the University of Aberdeen[1] with a binocular surface coil specifically designed for imaging the orbits.[2] Bilateral periorbital oedema was revealed with marked anterior nasal mucosal thickening and oedema (Fig. 2).

Treatment and progress

Systemic antihistamine therapy and an 8-week course of tetracycline (1 g per day) were ineffective. Oral prednisolone temporarily reduced the swelling but only at a dose >12·5 mg/day. Minocycline at a dose of 100 mg/day was commenced but discontinued after 6 weeks because it appeared to provoke a borderline photosensitivity at 400 nm. Oral isotretinoin 0·5 mg/kg was prescribed for 3 months without benefit. It was then decided to remove the implant. An incision was made vertically through the columella of the nose which was scarred. The strut of the hand-carved implant was removed and the cavity biopsied. The swab from this area also yielded a scanty growth of *S. aureus* on culture.

On histological examination it was noted that there was a mild, predominantly lymphocytic, inflammatory giant cell reaction in relation to amorphous granular material. Post-operatively there was no problem of infection. In the 2 years since his operation the oedema has not totally resolved but his condition is less troublesome.

Discussion

This was an unusual presentation of facial oedema. The differential diagnoses considered initially were solid facial oedema and lymphoedematous rosacea. Solid facial oedema has been noted as a complication of acne vulgaris,[3] usually occurring at least 4 years after the onset of the acne. The pathogenesis is so far unknown although it has been speculated that the chronic cutaneous inflammation from acne can produce lymphatic damage and subsequent progressive oedema of the face. Improvement after treatment with isotretinoin and clofazimine, both of which are known to stimulate macrophage function and phagocytosis, suggests an immunological response to the mediators of acne.[4] It has been reported that solid facial oedema in acne does not respond to antibiotics, but does respond to steroids in doses in excess of 15 mg/day.[5] Our patient did not respond to tetracycline and minocycline but was improved temporarily with oral prednisolone.

Similar inflammation, oedema and pilosebaceous changes occur in rare cases of rosacea.[6] Oedema of the face in rosacea may be similar to the acne/solid oedema syndrome but responds to antibiotic treatment.

The possible role of the prosthetic graft material in the aetiology of the facial oedema was considered. The adherence of bacteria to prosthetic graft material is thought to play an important role in the ultimate development of infections. It has been demonstrated that coagulase negative *Staphylococci* may be pathogenic in this situation.[7] Both *Escherichia coli* and *S. aureus* have been demonstrated to adhere to silicone, Proplast II, and Goretex.[8] Interestingly, in this study, the adherence of *S. aureus* was at a faster rate than *E. coli*, and the bacterial attachment continued to increase through the incubation time. This helps to explain the foreign-body effect of increased susceptibility to infection of foreign materials. Our patient had *S. aureus* isolated from nasal swabs and from the biopsy of the cavity after the removal of the silicone implant.

Long-term problems after insertion of silicone breast and joint prostheses have been recognized. Silicone lymphadenopathy has been noted in association with silicone mastitis[9,10] and after insertion of joint prostheses.[11-13] Silicone is now a well-recognized inducer of localized granulomatous inflammation but there are less common associations with more complex clinicopathological entities. Three patients developed unilateral proptosis 13–20 years after repair of orbital floor fractures with teflon implants.[14] A woman who had subcutaneous liquid silicone injections in the forehead for cosmetic reasons developed an acute inflammation of the eyelids and orbits 20 years later, which settled over 2–3 months with topical application of cortisone and heparin.[15] There is evidence of a death from a giant cell myocarditis associated with silicone rubber in dialysis tubing.[16] A silicone-induced foreign-body reaction with dental prostheses has also been reported.[17] Both scleroderma[18] and human adjuvant disease[19,20] have been noted after silicone augmentation mammoplasty. There are reports in which removal of the implanted prostheses led to some clinical improvement.[18] The mechanisms by which silicone can induce tissue damage have been reviewed.[21,22]

Our patient was assumed to have lymphoedematous rosacea, and solid facial oedema was also considered because of his resemblance to patients previously described.[2,3] There is, however, no doubt that the silicone-rubber implant was a crucial factor. The presence of local infection and the probability of migration of silicone particles causing a localized granulomatous inflammation, would appear to have resulted in his facial oedema. Modern radiological and bioengineering tests did not disclose this problem. Foreign-body implants should be added to the differential diagnosis of recurrent facial oedema in dermatological textbooks.

References

1 Redpath TW, Hutchison JMS, Eastwood LM *et al.* A low-field NMR imager for clinical use. *Journal of Physics E. Scientific Instruments* 1987; **20:** 1228–34.

2 Deans HE, Redpath TW, Smith FW *et al.* Magnetic resonance imaging of the orbits using a binocular surface coil. *Br J Radiol* 1988; **61:** 665–72.

3 Connelly MG, Winkelmann RK. Solid facial edema as a complication of acne vulgaris. *Arch Dermatol* 1985; **121:** 87–90.

4 Helander I, Aho HJ. Solid facial edema as a complication of acne vulgaris; treatment with isotretinoin and clofazimine. *Acta Derm Venereol (Stockh)* 1987; **67:** 535–7.

5 Camacho-Martinez F, Winkelmann RK. Solid facial edema as a manifestation of acne. *J Am Acad Dermatol* 1990; **22:** 129–30.

6 Marks R, Wilkinson DS. Rosacea and peri-oral dermatitis. In: *Textbook of Dermatology*, (Rook A, Wilkinson DS, Ebling FJG, Champion RH, Burton JR, eds), 4th edn. 1986; 1605–10.

7 Wong PTL, Nield DV, Khoo CTK. The pathogenicity of coagulase negative staphylococcus in the presence of silicone rubber implants. *Br J Plast Surg* 1987; **40:** 94–6.

8 Brewer AR, Stromberg BV. *In vitro* adherence of bacteria to prosthetic grafting materials. *Ann Plast Surg* 1990; **24:** 134–8.

9 Symmers WSC. Silicone mastitis in 'topless' waitresses and some other variations of foreign body mastitis. *B Med J* 1968; **3:** 19–22.

10 Truong LD, Cartwright J, Goodman MD, Woznicki D. Silicone lymphadenopathy associated with augmentation mammoplasty. *Am J Surg Pathol* 1988; **12:** 484–91.

11 Christie AJ, Weinberger KA, Dietrich M. Silicone lymphadenopathy and synovitis. Complications of silicone elastomer finger joint prostheses. *JAMA* 1977; **237:** 1463–4.

12 Jasin KA, Weerasinghe BD. Silicone lymphadenopathy, synovitis and osteitis complicating big toe silastic prosthesis. *J R Coll Surg Edin* 1986; **32:** 29–33.

13 Rogers LA, Longtine JA, Garnick MB, Pinkus GS. Silicone lymphadenopathy in a long distance runner: complications of a silastic prosthesis. *Hum Pathol* 1988; **19:** 1237–9.

14 Maurriello JA, Flanagan JC, Peyster RG. An unusual late complication of orbital floor fracture repair. *Ophthalmology* 1984; **91:** 102–7.

15 Hennekes R, Garus HJ. Late orbital complications following subcutaneous silicone injection. *Fortschr Ophthalmol* 1989; **86:** 249–50.

16 Kossovsky N, Cole P, Zackson DA. Giant cell myocarditis associated with silicone. An unusual case of biomaterials pathology discovered at autopsy using X-ray energy spectroscopic techniques. *Am J Clin Pathol* 1990; **93:** 148–52.

17 Acton C, Hoffman G, McKenna H, Moloney F. Silicone-induced foreign-body reaction after tempomandibular joint arthroplasty. *Aust Dent J* 1989; **34:** 228–32.

18 Spiera H. Scleroderma after silicone augmentation mammoplasty. *JAMA* 1988; **260:** 236–8.

19 Brozena SJ, Fenske NA, Cruse CW *et al.* Human adjuvant disease following augmentation mammoplasty. *Arch Dermatol* 1988; **124:** 1383–6.

20 Varga J, Schumacher HR, Jimenez SA. Systemic sclerosis after augmentation mammoplasty with silicone implants. *Am Intern Med* 1989; **111:** 377–83.

21 Travis WD, Balogh K, Abraham JL. Silicone granulomas: Report of three cases and review of the literature. *Human Pathol* 1985; **16:** 19–27.

22 Meigel W, Winzer M, Berg A, Wolff HH. Silikonom. *Z. Hautkr* 1989; **64:** 815–16.

British Journal of Dermatology (1991) **125**, 186–188.

ADONIS 000709639100193J

Palmoplantar and perioroficial keratoderma with corneal epithelial dysplasia: a new syndrome

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Accepted for publication 26 February 1991

Summary A case is reported of a male infant with congenital palmoplantar keratoderma and nail dystrophy who developed progressive perioral and perineal keratoderma. In addition, bilateral corneal epithelial dysplasia led to severe corneal scarring and impairment of vision. This syndrome has not been previously described.

Case report

A male infant was born at term (birth weight 7 lb 2 oz) after a normal pregnancy, to healthy unrelated parents. At birth he had abnormal nails, thickening of the palms and soles, scattered scaly patches on his face and a total alopecia. Dryness of the skin persisted and he was treated for recurrent cutaneous candidiasis.

At 6 months of age he had severe seborrhoeic eczema with cradle cap and crusted red patches on the face and shoulders which improved with topical treatment. He subsequently developed severe photophobia with red eyes and, at 13 months, was referred for ophthalmological assessment.

Examination revealed an atypical right-sided corneal ulcer and active vascularization. There was no evidence of vernal keratoconjunctivitis or infection and the presence of bilateral corneal epithelial dysplasia suggested a corneal dyskeratosis. Intensive therapy with topical methicillin, gentamicin and prednisolone 0·1% led to a gradual resolution of the ulcer but there was extensive residual corneal scarring of the right eye.

In addition to his eye disease, he had by then developed a grossly disfiguring keratoderma around the mouth and involving the whole perineum. The appearance was that of a macerated, infected, keratinous perioral plaque with fissuring at the corners of the mouth and in the thickened skin crease of the chin (Fig. 1). There was rugose thickening of the genital skin and a verrucous infected perianal plaque which extended on to the medial aspects of the thighs. He had a granular hyperkeratosis of the palms and soles and a severe nail dystrophy (yellow discoloration, thickening and subungual hyperkeratosis) of all finger and toenails. Additional features were extensive dry skin, encrusted and scaly

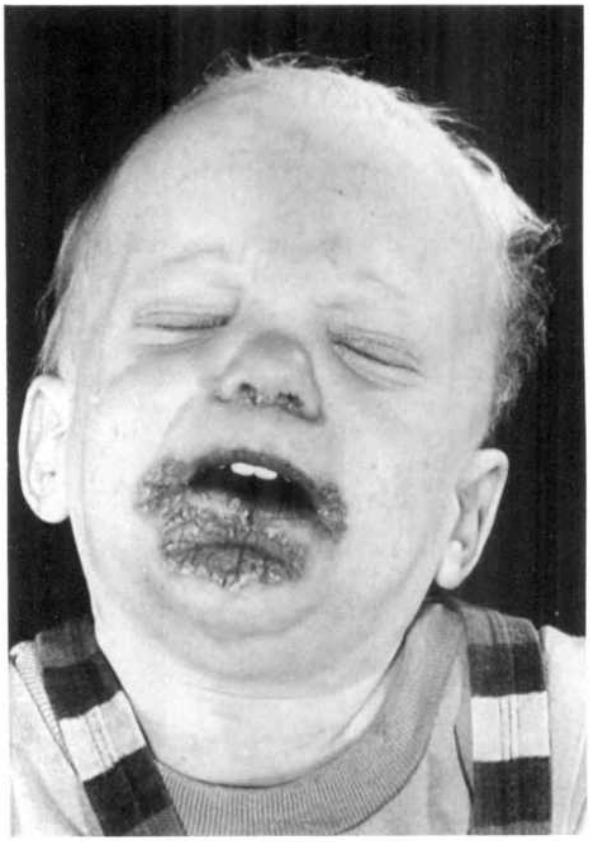

Figure 1. Keratinous perioral plaque with fissures, nasal crusting, blepharitis and sparse hair.

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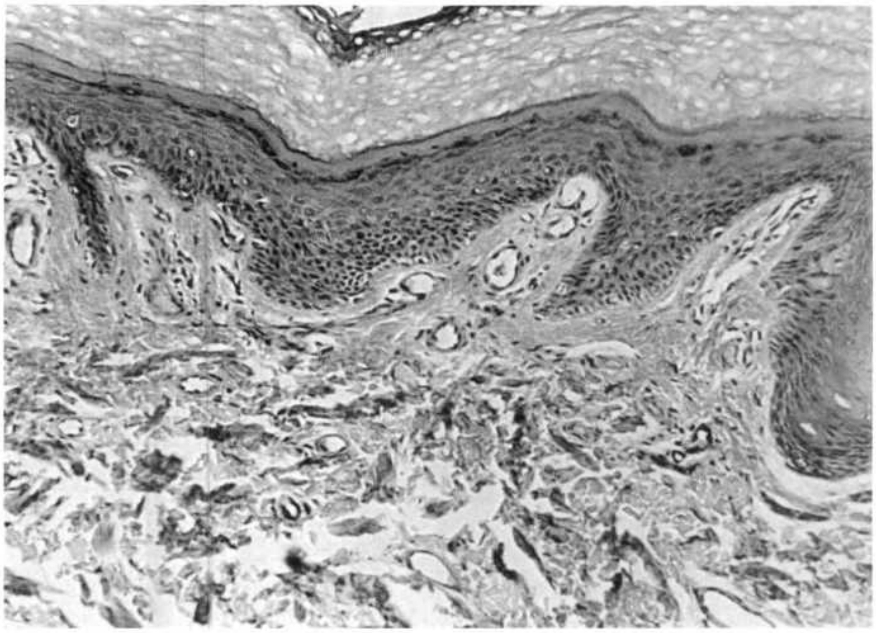

Figure 2. Biopsy from lateral heel (non-weight bearing) showing hyperkeratosis, mild acanthosis and preserved granular layer.

patches on the scalp with diffuse alopecia, sparse eyebrows and eyelashes and chronic blepharitis. His teeth were normal, specifically showing no enamel defects. Hearing tests were normal and his parents observed that sweating was normal.

His older sister had been treated for a vaginal rhabdomyosarcoma (sarcoma botyroides) at the age of 16 months and she was in full remission 2 years after cessation of chemotherapy and radiotherapy. Another sister was in good health and there was no family history of skin or genetic disorders.

Investigations

Full blood count, routine biochemical tests and chest X-ray were all normal. A sweat test yielded a sample of normal volume and electrolyte composition. Skin swabs on culture grew *Staphylococci*, *Pseudomonas aeruginosa* and *Candida albicans* and swabs from the eyes repeatedly yielded mixed organisms similar to those on skin swabs. Nail clippings failed to show any evidence of fungal infection. Immune function studies, including immunoglobulin and complement levels, T-cell subsets, PHA stimulation and candida killing, were normal.

A skin biopsy from the lateral heel (non-weight bearing skin) showed marked hyperkeratosis without parakeratosis, mild acanthosis and an intact granular layer (Fig. 2). The dermis was normal. Hair analysis showed no structural defect on light microscopy.

Progress

At 2·5 years the perioral and perianal keratoderma had become more pronounced and were frequently infected. He required repeated antibiotic and anticandidal therapy together with regular potassium permanganate soaks and intermittent use of povidone–iodine or hydrogen peroxide cream. The palmoplantar hyperkeratosis was unchanged, scalp hair remained sparse and the nail dystrophy had worsened. Severe chronic blepharitis and otitis externa persisted despite intensive therapy.

In addition, he had numerous small keratotic papules on the limbs and trunk and a biopsy of one of these lesions showed histological changes similar to the previous skin biopsy, with the additional feature of vacuolation in the basal keratinocytes. The bilateral corneal dysplasia had worsened and resulted in significant loss of vision. His growth continued along the 10th percentile and neurological development was normal.

Etretinate therapy, at a dose of 0·5 mg/kg/day, was started and this resulted in a noticeable improvement in his overall condition. The periorificial keratoderma was less pronounced, no further cutaneous or ocular infections have occurred and the blepharitis has settled.

Discussion

In the differential diagnosis, various disorders of keratinization were considered, the main contenders being the

KID (keratitis, ichthyosis, deafness) syndrome, ichthyosis follicularis and keratosis follicularis decalvans. Deafness was not a feature in this patient and the skin lesions were atypical for KID syndrome.[1] In ichthyosis follicularis, widespread follicular keratoses and focal hyperkeratoses are associated with a non-scarring alopecia and photophobia.[2] Keratosis follicularis spinulosa decalvans describes the association of severe keratosis pilaris with palmoplantar hyperkeratosis, corneal dystrophy and alopecia.[3] In neither of these conditions does periorificial keratoderma occur.

The periorificial lesions, although atypical, suggest the possibility of acrodermatitis enteropathica. His zinc level was at the lower end of the normal range but, apart from coinciding with an improvement in his mood, treatment with high-dose zinc supplements (Z span capsules) for 3 months did not influence the skin lesions.

The combination of hair, skin, nail and corneal abnormalities raised the possibility of hidrotic ectodermal dysplasia, dyskeratosis congenita or pachyonychia congenita but these disorders are not associated with perioral or perianal keratoderma.[4]

Of the congenital palmoplantar keratodermas, we could exclude the main types. Unna–Thost syndrome is a localized condition and epidermolytic hyperkeratosis shows striking histological changes. Tyrosinaemia type 2 (Richner–Hanhart syndrome) causes dendritic corneal ulcers but has a late onset with focal and painful palmoplantar keratoderma.[5] Subsequently we confirmed that our patient's amino-acid profile was normal. The absence of periodontal disease ruled out Papillon–Lefèvre syndrome and a mutilating keratoderma such as Vohwinkel's or mal de Meleda was not considered likely.

However, we were struck by the resemblance of our patient's features to the perioral and perineal lesions of Olmsted's syndrome. There have been four reported cases of Olmsted's syndrome including the original description by Olmsted in 1927.[6–9] All had a mutilating congenital palmoplantar keratoderma, striking periorificial keratinous plaques, nail dystrophy and scattered keratotic papules. Ocular complications were not a feature of these cases except for a unilateral chronic dacryocystitis in Olmsted's patient. Apart from an apparent autosomal dominant inheritance in Atherton's patients (a mother and son),[9] the available data suggest a sporadic occurrence of this disorder.

In spite of the similarities, the absence of a mutilating keratoderma and the presence of serious ocular dysplasia imply that our patient does not have Olmsted's syndrome. No doubt local factors aggravated the corneal disease but evidence of primary epithelial abnormality existed.

Standard topical treatments, antibiotics, zinc and biotin supplements were ineffective and the corneal disease was progressive. After due consideration of the possible ocular toxicity of systemic retinoid therapy, etretinate was started and after 4 months of treatment, both the skin and eye disease were markedly improved.

In summary, we report the clinical details of a male infant with congenital, non-mutilating palmoplantar keratoderma, severe perioral and perianal keratoderma, nail dystrophy, sparse hair, blepharitis and corneal dysplasia with scarring. There are certain similarities to Olmsted's syndrome, but the additional cutaneous and ocular features suggest a previously unknown ectodermal disorder.

References

1 Skinner BA, Greist MC, Norris AL. The keratitis, ichthyosis, deafness (KID) syndrome. *Arch Dermatol* 1981; 117: 285–9
2 Eramo LR, Esterly NB, Zieserl EJ *et al.* Ichthyosis follicularis with alopecia and photophobia. *Arch Dermatol* 1985, 121: 1167–74
3 Rand R, Baden HP. Keratosis follicularis spinulosa decalvans. *Arch Dermatol* 1983; 119: 22–6.
4 Freire-Maia N, Pinheiro M. *Ectodermal Dysplasias: a Clinical and Genetic study*, 1st edn. New York. Alan R Liss, Inc, 1984.
5 Fraser NG, MacDonald J, Griffiths WAD. Tyrosinaemia type 2 (Richner–Hanhart syndrome): report of two cases treated with etretinate. *Clin Exp Dermatol* 1987; 12: 440–3.
6 Olmsted HC. Keratodermia palmaris et plantaris congenitalis *Am J Dis Child* 1927; 33: 757–64.
7 Costa OG. *Acrokeratoses*. Minas Geraes, Brazil; Belo Horizonte, 1962.
8 Poulin Y, Perry HO, Muller SA. Olmsted syndrome—congenital palmoplantar and periorificial keratoderma. *J Am Acad Dermatol* 1984; 10: 600–10.
9 Atherton DJ, Sutton C, Jones BM. Mutilating palmoplantar keratoderma with periorificial keratotic plaques. *Br J Dermatol* 1990; 122: 245–252.

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Effect of oral isotretinoin therapy on saliva volume and composition

SIR, Isotretinoin is an effective treatment for acne vulgaris, but approximately 30% of patients receiving isotretinoin complain of a dry mouth.[1] About 10% describe a subjective change in the texture of the oral mucosa 'like having a thick layer of petroleum jelly applied throughout the mouth',[1] and some of our patients have said their saliva appears thick and viscous. We have therefore studied the effect of isotretinoin therapy on the volume and composition of saliva.

Six patients with severe acne (five male, one female, age range 13–28 years, mean 20 years) were treated with a mean daily dose of isotretinoin of 60 mg (range 30–90 mg). Each patient expectorated (instead of swallowing) whole saliva under basal conditions into a collection pot for exactly 1 h, before and after receiving oral isotretinoin for 1 month. Sodium azide, 8 mg, was added as an antimicrobial agent. The volume of each sample was measured and then dialysed exhaustively against distilled water and freeze dried (non-diffusible glycoconjugate). The dried material was weighed and subjected to proteolytic digestion,[2] centrifuged at 38,000 *g* for 30 min at 4°C and the supernatant applied to a column (2·4 × 61 cm) of Sephadex G150 and eluted with 0·15 M sodium chloride. The effluent was monitored for the presence of hexose[3] and the hexose-containing peaks were pooled according to the profile to give three fractions designated S_1, S_2 and S_3. The S_1 fraction was excluded from the column and represented glycopolypeptide material. This fraction was dialysed against distilled water and freeze dried.

The carbohydrate content of the the non-diffusible glycoconjugates and the glycopolypeptides were determined by gas–liquid chromatography.[4]

Table 1. The effect of oral isotretinoin on whole saliva ($n = 6$)

	Before isotretinoin	After isotretinoin
Non-diffusible glycoconjugates		
Volume (ml/h)	39·6 ± 13·7	37·1 ± 12·0
Weight (mg/h)	122·1 ± 26·0	101·4 ± 23·8
Concentration (mg/ml)	3·20 ± 0·66	2·81 ± 0·59
Carbohydrate content (nmol/mg)		
Fucose	208 ± 96	162 ± 40
Mannose	65 ± 19	66 ± 20
Galactose	360 ± 150	276 ± 87
Glucose	262 ± 116	94 ± 27
N-Acetylglucosamine	303 ± 105	234 ± 44
N-Acetylgalactosamine	164 ± 68	126 ± 32
Sialic acid	56 ± 23	38 ± 21
Carbohydrate (g/100 g)	28·4	20·0

Results expressed as mean values ± standard deviation.

There was no significant difference in the volume of saliva collected before and after 1 month of isotretinoin therapy (Table 1). Both the total amount and the carbohydrate content of the non-diffusible glycoconjugate content of saliva were reduced after isotretinoin, although neither trend reached significance (Table 1, Fig. 1). The major contaminants at this stage are proteins and non-mucous glycoproteins. These are susceptible to proteolysis and are therefore largely removed during exhaustive proteolysis and dialysis. Mucus glyproteins, however, are relatively resistant to proteolysis and remain as high-molecular-weight glycopolypeptides[5] and are therefore excluded from a column of Sephadex G150. Analysis of the glycopolypeptide fraction showed considerable enrichment of the carbohydrate content (Table 2). The carbohydrate content of this material was increased after isotretinoin

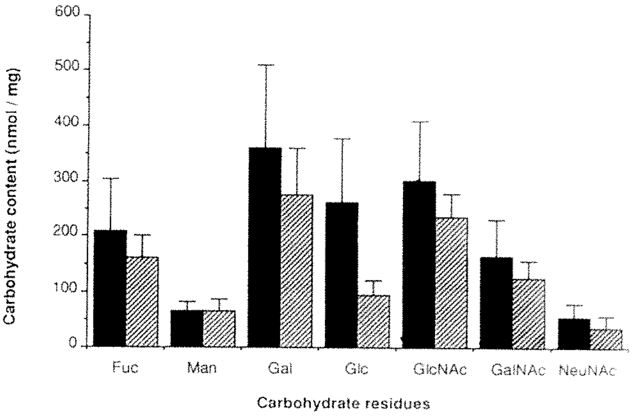

Figure 1. Carbohydrate content of non-diffusible glyconjugates of whole saliva before (■) and after (▨) isotretinoin. Values are expressed as means ± SD. Fuc, Fucose; Man, mannose; Gal, galactose; Glc, glucose; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; NeuNAc, N-acetylneuraminic acid.

Table 2. The effect of oral isotretinoin on the glycopolypeptide fraction from whole saliva

	Before isotretinoin	After isotretinoin
Glycopolypeptide weight/h	9·3 ± 3·8	9·1 ± 3·5
Carbohydrate content (nmol/mg)		
Fucose	792 ± 176	949 ± 200
Mannose	41 ± 15	55 ± 14
Galactose	1121 ± 253	1258 ± 281
Glucose	101 ± 39	223 ± 162
N-Acetylglucosamine	862 ± 279	1035 ± 205
N-Acetylgalactosamine	433 ± 164	458 ± 66
Sialic acid	51 ± 26	93 ± 59
Carbohydrate (g/100 g)	68	77

Results expressed as mean values ± standard deviation.

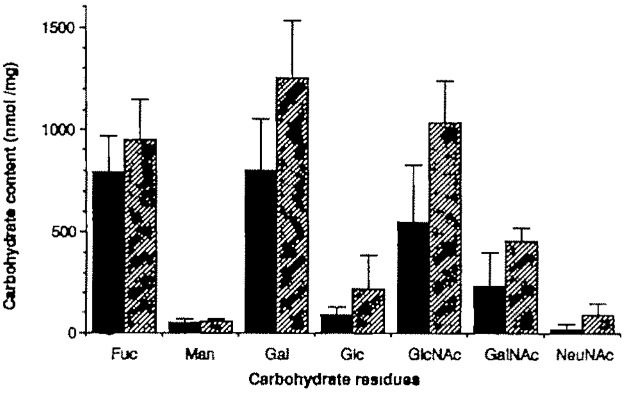

Figure 2. Carbohydrate content of glycopolypeptide fraction of whole saliva before (■) and after (▨) isotretinoin. Values are expressed as means ± SD. See Figure 1 for key to carbohydrate residues.

therapy but this trend did not reach significance (Table 2 and Fig. 2).

Although no significant reduction occurred in the non-diffusible glycoconjugate content of saliva after isotretinoin therapy, there was a definite trend towards a lower carbohydrate content. However, the glycopolypeptide fraction showed a trend towards an increased carbohydrate content after isotretinoin. The lower carbohydrate content in the non-diffusible glycoconjugate may be accounted for by an increase in the protein component of the non-mucus glycoproteins or the presence of other protein contaminants. An increased carbohydrate content of the glycopolypeptide fraction may result from larger oligosaccharide units within the salivary mucus glycoproteins. Alternatively isotretinoin might affect the number of carbohydrate units within salivary glycoproteins.

Salivary secretions contain a number of substances in addition to mucus glycoproteins such as other glycoproteins, proteins, immunoglobulins and enzymes, principally amylase. Secretions from the parotid, submandibular and sublingual salivary glands make up 90% of the whole saliva. Secretions from the accessary salivary gland of the soft palate, lips and cheeks together with desquamating cells and cellular debris make up the remainder. The saliva secreted by the three principal salivary glands differs in composition. The parotid contains mainly serous or seromucous acini, the submandibular predominantly seromucous with some mucous acini, and the sublingual mainly mucous acini. Thus the viscosity of saliva secreted by these three glands differs considerably, sublingual saliva being 11 times more viscous than parotid saliva. There are, furthermore, elaborate mechanisms controlling the composition, quantity and rate of salivary excretion.

Although we found no significant differences in the glycopolypeptide content of saliva collected before or after isotretinoin we cannot exclude an influence of the composition of saliva secreted by one particular gland. Cannulation of the individual salivary ducts would provide a more accurate picture of the salivary excretion from one gland. Our results suggest, however, that no major changes in salivary volume or glycoproteins occur after isotretinoin therapy, and the minor changes we observed are probably due to an increase in the non-mucous material in the saliva, possibly secondary to a change in epidermal cell turnover rate.

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References

1 Windhorst DB, Nigra T. General clinical toxicology of oral retinoids. *J Am Acad Dermatol* 1982; 6: 675–82
2 Fraser D, Clamp JR. The glycoprotein content of meconium. *Clin Chim Acta* 1975; 59: 301–7.
3 Dubois M, Gilles KA, Hamilton JK *et al.* Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 1956; 28: 350–6.
4 Clamp JR. A gas liquid chromatographic approach to the analysis of carbohydrates. *Biochem Soc Transact* 1977; 5: 1693–5.
5 Gibbons RA. Mucus of the mammalian genital tract. *Br Med Bull* 1978; 34: 34–8.

ADONIS 000709639100194W

Lupus, hereditary angioneurotic oedema and the risks of danazol treatment

Sir, We were interested to read the report by Duhra *et al.*[1] of a case of discoid lupus erythematosus associated with hereditary angioneurotic oedema. Although in this patient both diseases responded to treatment with danazol, in our two patients[2,3] therapy with danazol for 3 and 4 years caused the lupus-like eruptions to progress to typical subacute lupus erythematosus[4] with the appearance of anti-SSA/Ro antibodies in one of them and a lupus-like erythema in the other. In our first patient the cutaneous eruption cleared after stopping danazol, although a 10-year follow-up has shown that the anti-SSA/Ro antibodies have persisted. An exacerbation of a lupus erythematosus-like syndrome in a patient with non-C1-esterase-inhibitor-dependent angioedema on treatment with danazol has been reported.[5]

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References

1 Duhra P, Holmes J, Porter DI. Discoid lupus erythematosus associated with hereditary angioneurotic oedema. *Br J Dermatol* 1990; 123: 241–4.
2 Masse R, Youinou P, Dorval JC, Cledes J Reversal of lupus-erythematosus-like disease with danazol. *Lancet* 1980; ii: 651.
3 Youinou P, Dorval JC, Cledes J *et al.* A study of familial lupus erythematosus-like disease and hereditary anio-oedema treated with danazol. *Br J Dermatol* 1983, 108: 717–22.
4 Guillet G, Sassolas B, Plantin P *et al.* Anti-Ro-positive lupus and hereditary angioneurotic edema. A 7-year follow-up with worsen-

ing of lupus under danazol treatment. *Dermatologica* 1988; **177:** 370–5.
5 Fretwell MD, Altman LC. Exacerbation of a lupus-erythematosus-like syndrome during treatment of non-C1-esterase-inhibitor dependent angioedema with danazol. *J Allergy Clin Immunol* 1982; **69:** 306–10.

ADONIS 000709639100195B

Pseudoporphyria and sunbeds, a coincidence in identical twins?

SIR, We would like to report two further cases of pseudoporphyria due to use of UVA sunbeds that may shed some light on the mechanisms involved in the aetiology of this condition.

The two cases are monozygotic twin girls, aged 22 years who presented separately in Oxford and Chester. Both presented with the same symptoms of blistering and scarring on the backs of the hands for the previous 8 months (Fig. 1). The symptoms in each twin started within a few months of each other. Additional lesions had occurred on the cheeks, chest and ankles of one twin. The affected sites revealed scars with occasional small blisters or milia. One twin reported that one episode of blistering had occurred following sunburn. Both twins were taking the low-dose oral contraceptive pill, but no other medication. They both had black hair with very fair skin, compatible with skin type I.

In both centres the diagnosis was thought to be porphyria cutanea tarda. However, multiple measurements of porphyrins in blood, urine and faeces were negative, and there were normal levels of uroporphyrinogen decarboxylase. Coincidentally both were biopsied on the same day. The Chester twin had a subepidermal blister, with the basement membrane, as stained by PAS, in the floor of the blister. The Oxford twin also had perilesional skin biopsied to exclude a diagnosis of epidermolysis bullosa acquisita and direct immunofluorescence was negative.

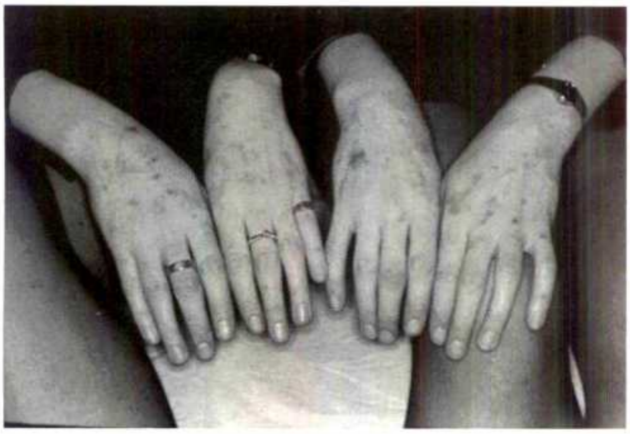

Figure 1. Hands of the twins showing the distribution of scars.

The diagnosis of pseudoporphyria was finally made and it was discovered that both had been using sunbeds extensively for over a year before their symptoms developed. The Oxford twin had used two UVA sunbeds at different times (Bolarium S® and RUVA®) for 30 min three times a week, in 2-monthly sessions over a period of 14 months, i.e. approximately 96 h in total. The Chester twin had used a separate UVA sunbed (Bolarium S® 'fast tan') for 30 min, five times per month for 8 months each year, and for 18 months longer than her twin, i.e. approximately 60 h in total. Both twins also spent much time sunbathing.

These cases of monozygotic twins with coincident development of sunbed-induced pseudoporphyria is strongly suggestive of a genetic factor in this condition. This genetic susceptibility is likely to be more than simply a common skin type, particularly in view of the timing of the development of the condition. In previous reports[1–5] a genetic factor has not been considered for this type of pseudoporphyria,[1–5] although porphyria cutanea tarda itself is inherited as an autosomal trait.[6]

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References

1 Epstein JH. Pseudoporphyria and UVA suntan salons. *Photochem Photobiol* 1987; **45:** 40S.
2 Farr PM, Marks JM, Diffey BL, Ince P. Skin fragility and blistering due to use of sunbeds. *Br J Med J* 1988; **296:** 1708–9.
3 Murphy GM, Wright J, Nicholls DSH *et al.* Sunbed-induced pseudoporphyria. *Br J Dermatol* 1989; **120:** 555–62.
4 Poh-Fitzpatrick MB, Ellis DL. Porphyria like bullous dermatosis after chronic intense tanning bed and/or sunlight exposure. *Arch Dermatol* 1989; **125:** 1236–8.
5 Stenberg A. Pseudoporphyria and sunbeds. *Acta Derm Venereol (Stockh)* 1990; **70:** 354–6.
6 Harber LC, Bickers DR. Porphyria and pseudoporphyria. *J Invest Dermatol* 1984; **82:** 207–9.

ADONIS 000709639100196O

Hydrochloric acid treatment of HEp-2 cells for the detection of masked anticentromere antibody (ACA)

SIR, We were interested to read your recent article on coexisting anticentromere antibody (ACA) and anti-topoisomerase I antibody (Topo I) in systemic sclerosis (SSc).[1] ACA and Topo-I had been considered to be mutally exclusive in SSc and prior to this only two patients with both antibodies have been previously described.[2] This group reported that 10 out of 180 patients with SSc were positive for both ACA and Topo I, using indirect immunofluorescence (IIF), double immunodiffusion (ID) and immunoblotting with recombinant antigens. They pointed out that the ACA pattern could not be recognized when these two antibodies coexisted due to a masking effect of Topo-I.

We wish to present a concise method of detecting coexisting ACA in sera identified positive for Topo I by ID. We observed that Topo-I antigens could be extracted by treatment with 0·1 N HCl for 30 min at room temperature, while centromere antigens were resistant to this treatment. Therefore, ACA which was masked by Topo I could be detected after treatment of HEp-2 cells with HCl.

Using both untreated HEp-2 cells and HCl-treated HEp-2 cells, we screened 47different sera found positive for Topo-I by the ID method. Only one serum produced both the fine speckled stainings for Topo I and the discrete speckled stainings for ACA on untreated HEp-2 cells. When HCl-treated HEp-2 cells were used as the substrate, this serum clearly showed the typical staining for ACA on mitotic cells. Of the 47 sera, only one was positive for ACA on HCl-treated HEp-2 cells.

Our study indicates that ACA and Topo I are mutually exclusive in Japanese patients with SSc, although they may rarely coexist in low titres. The use of HCl-treated HEp-2 cells provides a concise method for screening the coexistence of both antibodies.

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References

1 Jarzabek-Chorzelska M, Błaszczyk M, Kołacińska-Strasz Z *et al.* Are ACA and Scl 70 antibodies mutually exclusive? *Br J Dermatol* 1990; **122:** 201–8.

2 Ruffatti A, Calligaro A, Ferri C *et al.* Association of anti-centromere and anti-Scl 70 antibodies in scleroderma. Report of two cases. *J Clin Lab Immunol* 1985; **16:** 227–9.

ADONIS 0007096391001973

Melasma: a mask of stress

Sir, The pathogenesis of melasma is unknown, and a number of aetiological factors have been implicated that include oral contraceptives, pregnancy, cosmetics, nutritional factors, drugs, endocrine disorders and thyroid disease.[1-2] We wish to report two patients who developed melasma that was probably related to emotional stress.

The first was a 35-year-old woman who presented with a 2-month episode of symmetrical hyperpigmented macules on her cheeks. There was no other underlying factor, although there was a history of recent emotional stress in that her husband had died suddenly in an accident 2 months prior to the development of the hyperpigmentation on her face.

The second case was that of a 38-year-old unmarried woman with a 6-month history of hyperpigmentation of the skin on the cheeks and nose (Fig. 1). There was no obvious cause to account for her melasma and routine laboratory investigations and hormonal tests were all within the normal range. This patient was suffering from severe emotional stress because her brother had died 10 weeks before the development of the melasma.

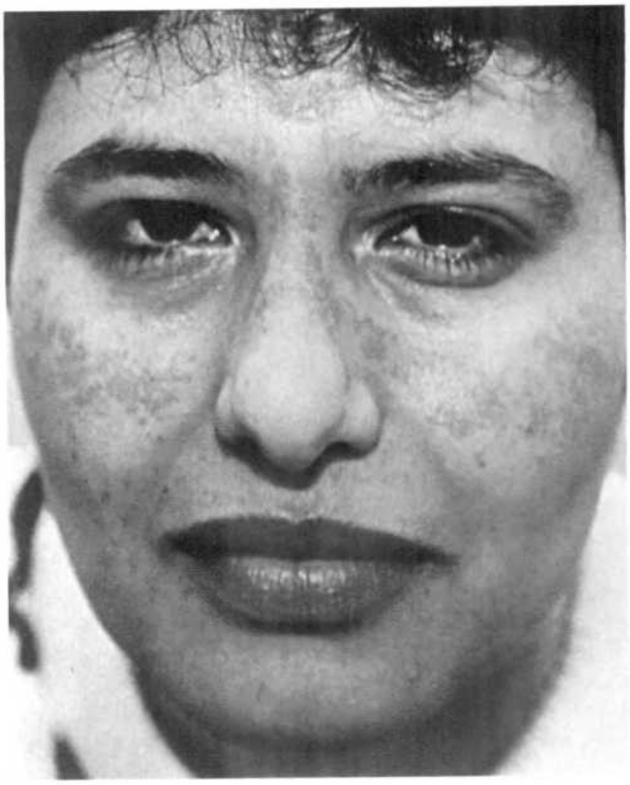

Figure 1. Case 2: melasma on face.

Both of our patients developed melasma 8–10 weeks following the sudden death of a close relative. There was no other factor in either case for their melasma, and previous pregnancies were not associated with the condition. Both of these women were of Sephardic origin (Jews of Spanish descent) and had dark skin. We believe that the appearance of the melasma folowing sudden emotional stress was not coincidental. The release of melanocyte-stimulating hormone (MSH) is controlled by the hypothalamus[3] which is known to be influenced by emotions. It is quite likely that emotional stress induces the release of MSH and in this way contributes to the development of the hyperpigmentation of the skin.

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References

1 Mosher DB, Fitzpatrick TB, Ortonne JP, Yoshiaki H. Disorders of pigmentation. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K *et al.*, eds), 3rd edn. New York: McGraw-Hill, 1987; 794–876.

2 Lurfi RJ, Fridmanis M, Misiunas AL *et al.* Association of melasma with thyroid autoimmunity and other thyroidal abnormalities and their relationship to the origin of the melanasma. *J Clin Endocrinol Metab* 1985; **61**: 28–31.
3 Taleisnik S, Orias R. An MSH-releasing factor in hypothalamus extracts. *Am J Physiol* 1965; **208**: 293–6.

ADONIS 000709639100198G

Foam cells after treatment with hydrocolloid dressings

SIR, Hydrocolloid dressings are commonly used in the treatment of ulcerations of the skin and provide many advantages.[1] However, in a recent paper Reuterving *et al.*[2] noted biochemical and histological changes in the granulation tissue of rats after treatment with a hydrocolloid dressing (Granuflex®, Squibb).

Macrophages underwent morphological alterations resembling those of foam cells and these were found in all wounds treated with the hydrocolloid dressing.[2] The hydrocolloid dressing used is composed of polyisobutylene (a hydrophobic synthetic polymer) and three types of hydrophilic particles: carboxymethylcellulose sodium, pectin and gelatin. It was suggested that one or more of the hydrophilic particles were phagocytosed by the macrophages resulting in these changes.[2]

To study the composite effect of the hydrophilic particles on wound healing, the breaking strength[3] of incisional wounds was examined on the tenth post-operative day. A total of 200 mg carboxymethylcellulose sodium, pectin and gelatin in equal proportions was introduced into one of two parallel 6-cm-long dorsal skin incisions on each of eight male Sprague–Dawley rats; the contralateral incision received no treatment (control). No significant difference (paired t-test, $n=8$) in breaking strength was found between experimental wounds (263 ± 25 g/0·5 cm; mean ± SEM) and control wounds (257 ± 25). However, no foam cells were found in the experimental wounds on light microscopy. We therefore carried out a study to find out which of the substance(s) in the hydrocolloid dressings was responsible for the presence of foam cells. Excisional full-thickness skin wounds on rats were treated for 10 days with either a single application of a complete hydrocolloid dressing, with only the hydrophilic particles or with the polyisobutylene alone. The hydrocolloid dressing or the hydrophilic particles alone disintegrated or dissolved in the wounds, whereas the polyisobutylene alone was unaffected macroscopically after treatment. Histological examination revealed granulation tissue abundant in foam cells but only in wounds treated with the hydrocolloid dressing. Foam cells were also absent if the wounds were treated with a polyurethane membrane (OpSite®, S&N).

Thus, it appears that the appearance of foam cells after hydrocolloid wound treatment in rats is due to the combination of one or more of the hydrophilic particles and the polyisobutylene and not to a single component of hydrocolloid dressings. It is possible that swelling of the hydrophilic particles in contact with wound exudate makes the polyisobutylene more available for phagocytosis by macrophages. The clinical implications of our finding are not clear. It may be that macrophages, cells that are essential for wound healing, are adversely affected by phagocytosis of the dressing material. However, wounds in both rats[2] and humans[1] treated with hydrocolloid dressings heal more rapidly than conventionally treated wounds.

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References

1 Falanga V. Occlusive wound dressings. Why, when, which? *Arch Dermatol* 1988; **124**: 872–7.
2 Reuterving C-O, Ågren MS, Söderberg TA *et al.* The effects of occlusive dressings on inflammation and granulation tissue formation in excised wounds in rats. *Scand J Plast Reconstr Surg* 1989; **23**: 89–96.
3 Ågren MS, Franzén L. Influence of zinc deficiency on breaking strength of 3-week-old skin incisions in the rat. *Acta Chir Scand* 1990; **156**: 667–70.

ADONIS 000709639100199T

No association between cicatricial pemphigoid and malignant disease

SIR, A recent study of host factors involved in the metastatic potential of cutaneous squamous cell carcinomas identified patients with cicatricial pemphigoid (CP) as being at high risk.[1] It was unclear in this report whether the increased frequency of malignancy associated with CP is intrinsic to the condition or related to the immunosuppressive therapy for the disease. Over the years, there have been sporadic reports of CP associated with malignant disease.[2-7] Among the neoplasms recorded are a variety of carcinomas, leiomyosarcoma of the small bowel and abdomen and chronic lymphocytic leukaemia. We have investigated as to whether patients with CP have an excess prevalence of malignant disease.

Thirty-four patients with CP were investigated. All cutaneous and internal malignant diseases occurring in the patients were ascertained by questionnaire and case note review in a retrospective manner. Each patient was matched with two controls (from biopsy clinics) and the prevalence of internal malignancy in the two groups compared. The controls were of the same sex and of an age within 6 months of the patient and none were known to have any dermatological conditions associated with internal malignancy. Two out of 34 patients (5·8%) had malignant disease compared to three out of 68 controls (4·4%). Using Fisher's exact test, this difference is not statistically significant (P=0·795). The two patients were both female who developed adenocarcinoma of the breast at the age of 56 and 69, respectively. Their neoplasms occurred 5 years after the onset of CP and did not run a parallel course to it. Neither patient had received oral corticosteroids or any other immunosuppressive therapy known to predispose to neoplasia during the course of their follow-up. One patient died from widespread metastasis 2 years after the diagnosis of cancer while the other continues to be well a year after simple mastectomy and is on tamoxifen. None of the CP patients had cutaneous malignancies.

Breast cancer is common in elderly females and its occurence in two of our patients is not unexpected. These observations are in accordance with Hardy *et al.*[2] who concluded that the increased frequency of malignancy in 12 out of 81 patients in their series was attributable to the advanced age of these patients. It is not surprising for some CP patients to subsequently develop malignancy as both are diseases that are more common in the elderly. Few cases of a strictly concurrent course between CP and tumour have been documented in the literature and this makes the association even more doubtful. Therefore, patients with CP do not appear to have a higher risk of developing systemic or cutaneous cancers. Further studies on larger series of CP patients will clarify the link between CP and malignancy.

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References

1 Dinehart SM, Chu DZJ, Maners AM *et al.* Immunosuppression in patients with metastatic squamous cell carcinoma from the skin. *J Dermatol Surg Oncol* 1990; **16:** 271–4.
2 Hardy KM, Perry HO, Pingree GC, Kirby TJ. Benign mucous membrane pemphigoid. *Arch Dermatol* 1971; **104:** 467–75.
3 Polliack A. Benign mucous membrane pemphigoid with laryngeal stenosis in a patient with thyroid carcinoma. *Arch Pathol* 1968; **86:** 48–51.
4 David M, Oren N, Feverman EF. Occurrence of cicatricial pemphigoid and leiomyosarcoma in a psoriatic patient. *Dermatologica* 1962; **170:** 256–9.
5 Chadfield HW, Kanagasundaram CR. Carcinoma in benign mucous membrane pemphigoid (ocular pemphigus). *BJ Dermatol* 1962; **74:** 458–61.
6 Greer KE, Beacham BE, Askew FC. Benign mucous membrane pemphigoid in association with internal malignancy. *Cutis* 1980; **25:** 183–5.
7 Kilby PE. Carcinoma of the pancreas presenting with benign mucous membrane pemphigoid. *Cancer* 1965; **18:** 847–50.

ADONIS 000709639100200S

Lichen planus of the nails with giant cells: lichen nitidus?

SIR, Nail changes may be the only manifestation of several dermatological disorders and although a biopsy of the nail may make it possible to correlate the pathology with a specific disease, sometimes this is not possible. We report a 62-year-old patient with a long-standing onychodystrophy with the pathological features that resembled both lichen planus and lichen nitidus.

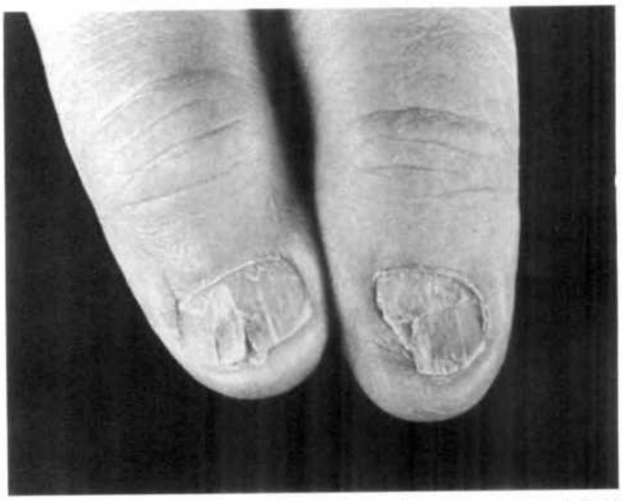
Figure 1. Marked onchodystrophy with thinning of the nails and distal splitting.

A 62-year-old female was referred to our department in 1989 with an onychodystrophy that affected the fingernails which were thinned with longitudinal ridging, splitting, distal notching and onycholysis (Fig. 1). Her toenails were normal. She was in good health and there were no other abnormalities. The patient had noted that the changes in the nail first occurred 15 years ago with spontaneous improvement and relapses. A longitudinal nail biopsy was performed on her left thumb and the histology showed a dense infiltrate in the superficial dermis of the proximal nail matrix. This band-like infiltrate consisted of lymphocytes, a large number of histiocytes and numerous multinucleate giant cells. The basal layer showed moderate hydropic changes but there was no hyperkeratosis, hypergranulosis or acanthosis of the epidermis (Fig. 2).

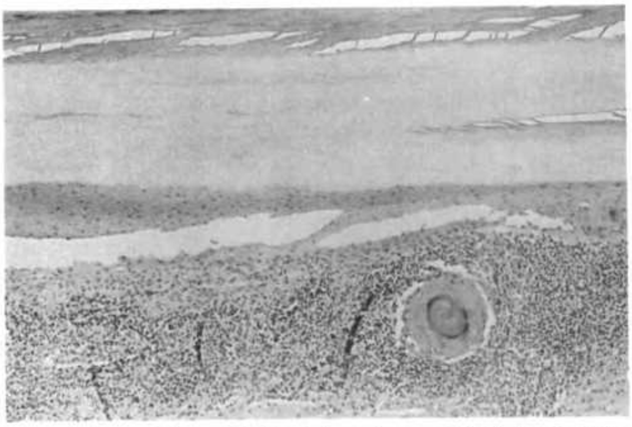
Figure 2. Dense lichenoid infiltrate involving superficial dermis of the proximal nail matrix (haematoxylin and eosin, × 125).

She was treated with intramuscular slow-release triamcinolone in a dosage of 40 mg every 2 weeks for 4 months. There was no improvement of her nails at 6 months follow-up.

Inflammatory dermatoses may show unusual pathological features in the nails that differ from usual skin pathology.[1] The histology in our patient showed features of both lichen planus and lichen nitidus.[2] Epithelioid and giant cells have been described to occur occasionally in the skin lesions of lichen planus,[3,4] and our case could be diagnosed as that of nail lichen planus with unusual granulomatous features. However, there is the possibility that the nail changes were that of lichen nitidus. Three cases of lichen nitidus with nail involvement have been reported.[5-7] The nail changes consisted of moderate pitting and rippling in one case[6] and roughness due to longitudinal ridging in the other two cases.[5,7] Swelling of the proximal nail fold was also present in one patient.[7] The relationship between lichen planus and lichen nitidus remains unclear and some authors have suggested that lichen nitidus represents a variant of lichen planus.[8-10] The unusual features of our patient with only nail involvement does support the view that the two conditions are closely related.

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References

1 Jerasutus S. Histology and histopathology. In: *Nails, Therapy, Diagnosis, Surgery* (Scher RK, Daniel CR, eds). Philadelphia: Saunders Company, 1990; 52–75.
2 Zaias N. *The Nail in Health and Disease.* Norwalk, Connecticut: Appleton Lange, 1990; 169.
3 Wilson HTH, Bett DCG. Miliary lesions in lichen planus. *Arch Dermatol* 1961; **83;** 920–3.
4 Ellis FA. Histopathology of lichen planus based on analysis of one hundred biopsy specimens. *J Invest Dermatol* 1967; **48:** 143–8.
5 Barker LP. Lichen nitidus, generalised, with nail changes. *Arch Dermatol* 1955; **72:** 487–8.
6 Kellett JK, Beck MH. Lichen nitidus associated with distinctive nail changes. *Clin Exp Dermatol* 1984; **9:** 201–4.
7. Natarajan S, Dick DC. Lichen nitidus associated with nail changes. *Int J Dermatol* 1986; **25:** 461–2.
8 Ellis FA, Hill WF. Is lichen nitidus a variant of lichen planus? *Arch Dermatol Syphilol* 1938; **38:** 568–73.
9 Gougerot H, Civatte A. Critéres cliniques et histologiques des lichen planus cutanés et muqueux: délimitation. *Ann Dermatol Syphilol* 1953; **80:** 5–29.
10 Stankler C. The identity of lichen planus and lichen nitidus. *Br J Dermatol* 1967; **79:** 125–6.

ADONIS 000709639100201L

British Journal of Dermatology (1991) 125, 196–197.

Book Reviews

Oxford Textbook of Functional Anatomy, Volume 3, Head and Neck. P.MacKinnon and J.Morris (1990). Oxford: Oxford University Press. Pp. 154. ISBN 0-19-261519-X. Price £15.00.

This book is the third part of a three-volume series of functional anatomy, the other two volumes having dealt with the musculo-skeletal system and the abdomen and thorax. All books in this series aim to emphasize the functional and living anatomy—and they succeed.

After a brief introduction there is an enlightening chapter on how to think about the tissues you are studying. The authors encourage the reader that whilst they are looking at a tissue and a structure they should try and think what is the structure doing there and why is it in that particular position? The bulk of the book is then set out in a series of 'seminars'. The authors claim you should spend two or more hours on each seminar. Each seminar, of which there are fifteen, is set out in a similar manner with sections of developmental anatomy, living anatomy and a detailed description of a prosection and, where relevant, radiological appearances. Each seminar is skillfully illustrated with high calibre line drawings, X-rays and high quality colour clinical photographs. Throughout each seminar there are dotted questions which encourage thinking about the subject under study.

For a dermatologist, and in particular a dermatological surgeon, this book goes into much more detail than is necessary but, for the interested clinician wishing a rapid update on their anatomical knowledge, this book is an excellent way of revising the position of vital structures. It offers a very thorough and pleasant way of learning a usually difficult subject. It will be of great benefit to medical students, dental students, and primary F.R.C.S. candidates. I wish I could have had this kind of book as an undergraduate.

S.I.White

Methods for Skin Absorption. Edited by B.W.Kemppainen and W.G.Reifenrath (1990). CRC Press: Boca Raton; Ann Arbor; Boston; USA. Pp. 219. ISBN 0-8493-4651-7. Price £108.

The book is intended for laboratory scientists, 'conceived in the spirit of the laboratory visit' to assess existing methods and to describe reliable procedures. It achieves its objectives and is an up-to-date critical assessment of methods to examine percutaneous absorption, their limitations and applications.

There are 12 chapters describing *in-vitro* and *in-vivo* methods to study and assay skin penetration, properties and physical characterization of substances influencing absorption, procedures to detect penetration and finally the validity of absorption and metabolic studies when related to *in-vivo* exposure.

An excellent chapter by Stephen Frantz compares the diffusion cells available for *in vitro* skin absorption studies, including that of Thomas Franz which is a model on which other designs are based, and describes in detail their components and assembly with a list of suppliers. There is useful guidance on the suitability of materials for the chambers and comments on appropriate test volumes and temperature. The following chapter by G.S.Hawkins overlaps the former, but provides detailed critical descriptions of operating procedures with a further list of suppliers of equipment, all American. A chapter on detection methods, with brief descriptions of the essentials of separation procedures, calibration and biological assays, complements descriptions of the absorption techniques.

Four chapters describe the essentials of *in-vivo* penetration studies on animals, their care, methods of application and the techniques to identify and assay penetration. One of the longer chapters by L.L.Hall and P.V.Shah describes the methods to detect absorption and to study metabolism. There are few descriptions specifically on human tests, and a caveat on the ethical considerations.

The work is a valuable practical guide to all those concerned with laboratory studies on skin absorption and metabolism. There is some discussion on the physics of penetration, but little on the skin physiology controlling penetration and metabolism, but in fairness the book was not conceived for this purpose.

W.E.Parish

Dermatology. M.Orkin, H.I.Maibach and M.V.Dahl (1991). Hemel Hempstead: Prentice-Hall International Inc. Pp. 696. ISBN 0-8385-1300-X. Price £32.50.

This compact dermatology text book has 65 contributors (predominantly from the United States) and is aimed at an audience ranging from ancillary medical staff and nurses to medical students, general physicians and dermatologists.

It is a well-edited book and would be a useful reference source up to and including trainee dermatologists. There is a comprehensive and acurate index and the book itself is laid out with a uniformity of style which makes it easy to read. It is perhaps ideally suited for dermatology SHOs and registrars who would find it a reasonably comprehensive and up-to-date introduction to the subject of dermatology.

The book is split into 11 sections, the first of which deals with the structure and properties of normal skin. There follows an excellent section on dermatological diagnosis and the chapter on dermatopathology is particularly informative and helpful for those just starting out in dermatology, explaining the terminology and illustrating the major histopathology patterns with excellent black-and-white photographs. The chapters on immunofluorescence and electromicroscopy, although brief, give the essential background information. A few further references are given at the end of each chapter.

The third section of the book deals with infections and infestations. This is, again, reasonably comprehensive, but it is perhaps a pity that there were not more illustrations or photographs for all the clinical chapters.

The penultimate section of the book deals with skin disorders affecting specific populations focusing, for example, on disorders of children or skin diseases in Negros. The final chapter deals with therapeutics and skin surgery and maintains the generally high standard present throughout the whole of the book.

Being just a one-volume compact textbook it is inevitable that many aspects of dermatology can only be touched on in a relatively superficial manner. Colour photographs would, I think have been better and further references would give the text more authority.

At £32.50, however, this book is undoubtedly good value for money coming somewhere between a comprehensive primer and a mini textbook. It would certainly be useful for those starting their training in dermatology and for others wanting an accessible and affordable dermatology reference source.

J.Wilkinson

Books received

Raynaud's Syndrome. Edited by E.D.Cooke, J.M.Porter and A.N.Nicolaides (1991). London: Med-Orion Publishing Company. Pp. 230. Illustrations 73. ISBN 9-9635925-1-1. Price £49.00 HB.

Investigation of Patients with Deep Vein Thrombosis and Chronic Venous Insufficiency. A.N.Nicolaides and D.S.Sumner (1991). London: Med-Orion Publishing Company. Pp. 80. Illustrations 46. ISBN 9-9635925-0-3. Price £28.50 PB.

Year Book of Dermatology 1990. Edited by A.J.Sober and T.B.Fitzpatrick (1990). St Louis: Mosby Year Book. Pp. 496.

The *Year Book* needs no review and the current editors can be congratulated on their choice of material to abstract. The opening article is by Dr T.B.Fitzpatrick and is on ozone depletion and the dermatologist. The following section of statistics of interest to the dermatologist is mainly of relevance to those in the United States. In the future I would like the authors to compile a similar set of European statistics.

Rona M.MacKie

Cosmetic and Medical Electrolysis and Temporary Hair Removal. R.N.Richards and G.E.Meharg (1991). Ontario: Medric Ltd. Pp. 480. ISBN 0-9694746-0-1. Price $59.95 US + postage & handling.

British Journal of Dermatology (1991) 125, 198.

News and Notices

Second International Congress on Ultrasound and the Skin, 18–21 September 1991, Modena, Italy

The Second International Congress on Ultrasound and the Skin will be held at the University of Modena on 18–21 September 1991. For further information contact: the Organizing Secretariat, Planning Congressi, Via Crociali 2, 40138 Bologna, Italy. Telephone: 39-51-302980.

6th International Conference on Behçet's Disease, 30 June–1 July 1993, Paris

The 6th International Conference on Behçet's Disease will be held in Paris on 30 June–1 July 1993. There will be plenary sessions, free papers and poster sessions and these will cover all aspects of Behçet's disease and particularly the adequacy of new criteria.

In honour of the centenary of Charcot, the meeting will be held at the Pitié-Salpêtrière Hospital. Pharmaceutical company exhibits will be on display in the seventeenth century chapel built by Le Vau. The deadline for abstracts is 1 February 1993. For further information contact: Professor Bertrand Wechsler, Pitié-Salpêtrière Hospital, 47/83 Bd de l'Hôpital, 75013 Paris Cedex 13, France. Telephone: (1)-45-70-26-67.

4th European Symposium on Psoriasis, 16–18 September 1993, Trieste

The 4th European Symposium on Psoriasis will be held in Trieste on 16–18 September, 1993. For further information contact: Dr F.Kokelj, Clinica Dermatologica, c/o Ospendale di Cattinara, Strada di Fiume, 34149 Trieste, Italy. Telephone: 040-7764401.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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picogram(s) (10^{-12}g)	pg
hours(s)	h
minute(s)	min
centimetre(s)	cm
second(s)	s
cubic millimetre(s)	mm^3
millimetre(s)	mm
millicurie(s)	mCi
milliequivalent	mmol
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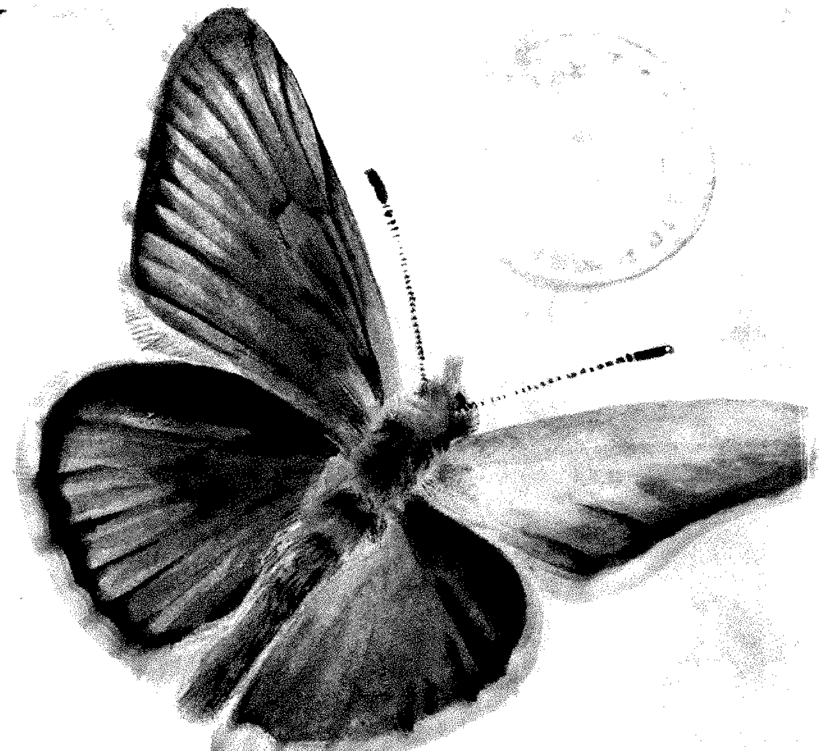

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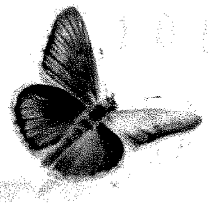

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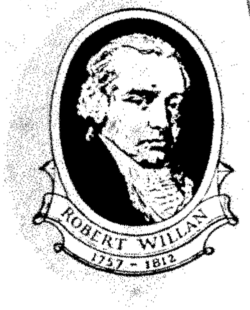

British Journal of Dermatology

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British Journal of Dermatology

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SUBSCRIPTION INFORMATION. *British Journal of Dermatology* is published monthly (2 volumes per annum) and the subscription prices for 1991 are £119.50 (U.K.), £130.00 (overseas except North America), $237.00 (U.S.A. and Canada), in all cases post free. Orders for current subscriptions and back issues should be sent to Journal Subscriptions Dept., Marston Book Services, P.O. Box 87, Oxford, U.K. Tel. 0865 791197, Telex 837515 Mardis G. Fax 0865 721205, all other business correspondence, including orders for offprints and advertising space, should be addressed to Blackwell Scientific Publications Ltd., Osney Mead, Oxford OX2 0EL (telephone no. 0865 240201, Telex 83355 Medbok G. Fax 0865 721205).

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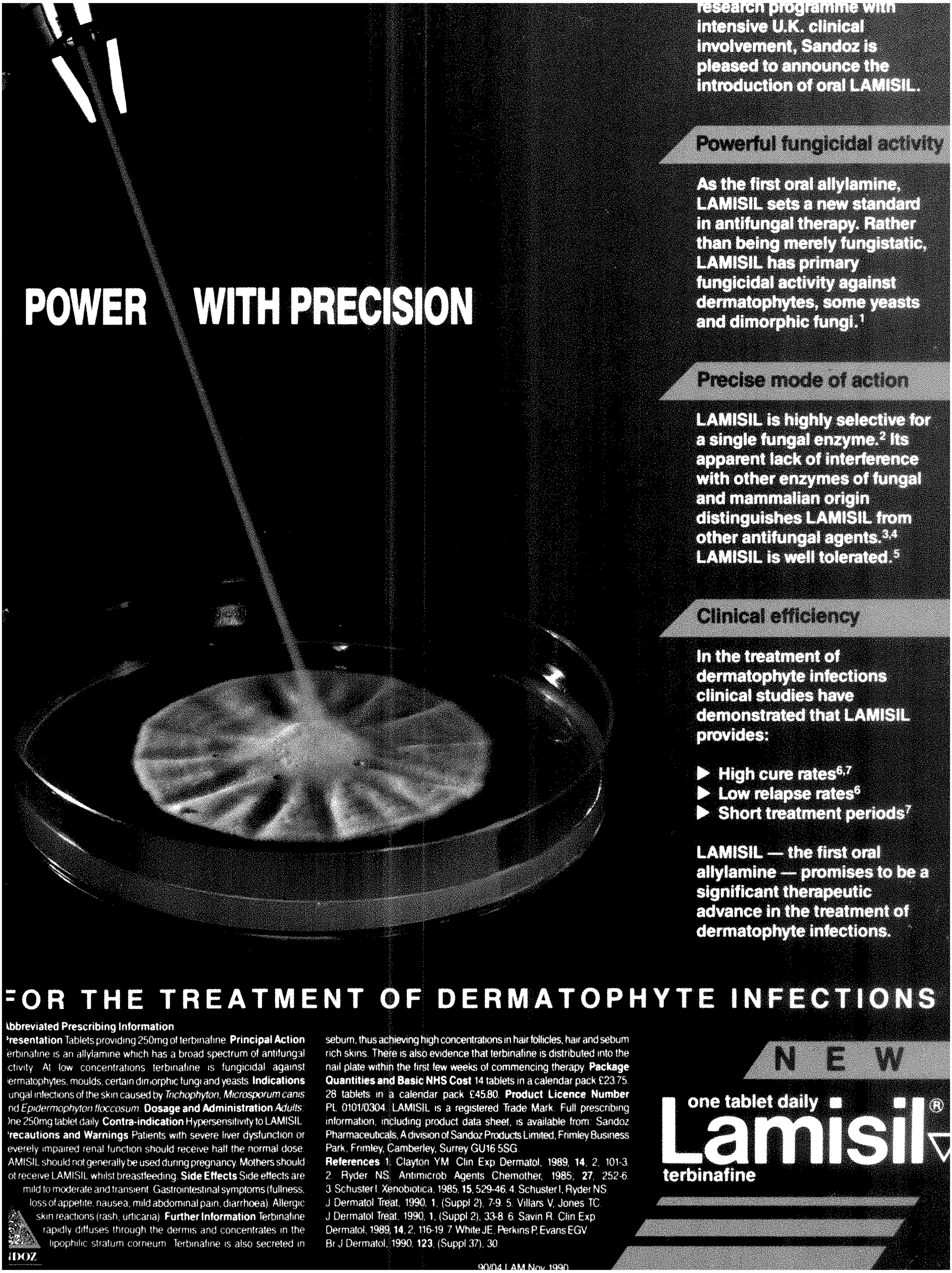

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References 1. Clayton YM. Clin Exp Dermatol, 1989, **14**, 2, 101-3. 2. Ryder NS. Antimicrob Agents Chemother, 1985; **27**, 252-6. 3. Schuster I. Xenobiotica, 1985, **15**, 529-46. 4. Schuster I, Ryder NS. J Dermatol Treat, 1990, 1, (Suppl 2), 7-9. 5. Villars V, Jones TC. J Dermatol Treat, 1990, 1, (Suppl 2), 33-8. 6. Savin R. Clin Exp Dermatol, 1989, **14**, 2, 116-19. 7. White JE, Perkins P, Evans EGV. Br J Dermatol, 1990, **123**, (Suppl 37), 30.

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Editors: Nancy B. Esterly, M.D.; Childrens Hospital of Wisconsin
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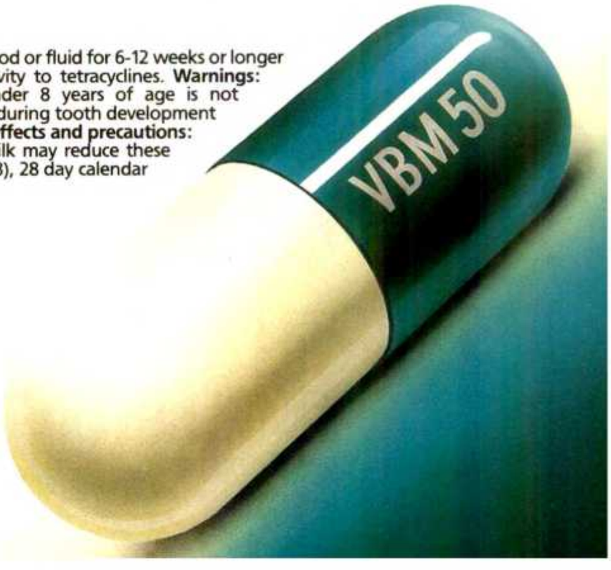

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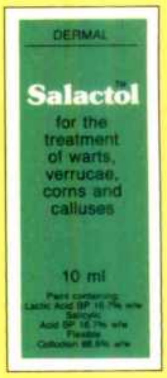

British Journal of Dermatology (1991) **125**, 199–210. ADONIS 000709639100202Y

REVIEW

The antiphospholipid syndrome. Clinical correlations, cutaneous features, mechanism of thrombosis and treatment of patients with the lupus anticoagulant and anticardiolipin antibodies

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Accepted for publication 2 May 1991

The lupus anticoagulant

In 1952, Conley and Hartmann described a unique *in-vitro* anticoagulant phenomenon occurring in two patients with systemic lupus erythematosus (SLE) and a haemorrhagic disorder.[1] The anticoagulant prolonged the whole blood clotting time and the prothrombin time, but no specific clotting factor deficiency was detectable. The term 'lupus anticoagulant' was introduced in 1972 to describe this phenomenon,[2] which was shown to be due to the presence of an inhibitor directed against negatively charged phospholipid in the clotting cascade. It is now known that the lupus anticoagulant (LA) is an IgG or IgM antibody that interferes with the formation of the prothrombinase complex composed of factor X, factor V, phospholipid and calcium. Thus, there is inhibition of conversion of prothrombin to thrombin during the final stages of coagulation (Fig. 1).[3]

Detection of a lupus anticoagulant

In vitro, the LA can be demonstrated in the plasma by prolongation of the phospholipid-dependent coagulation tests. The activated partial thromboplastin time (APTT) is characteristically prolonged, with a slightly prolonged prothrombin time (PT) in some patients. The thrombin time (TT) is normal. The abnormalities in PT and APTT are not corrected by the addition of an equal volume of normal plasma as would be expected in a clotting factor deficiency. The presence of the LA also produces a characteristic prolongation of PT when this test is carried out using dilute thromboplastin, even when the standard PT is normal. Two further tests are frequently used, the dilute Russel viper venom time[4] and Exner's test.[5] Russel viper venom contains enzymes that activate clotting factors V, IX and X in the presence of calcium and phospholipid. In the presence of a LA the clotting time is prolonged, but corrected when phospholipid is replaced by activated platelets. In Exner's test, the KCT of various dilutions of patient's plasma in normal plasma is plotted against the percentage of patient's plasma. The shape of the resultant curve is used to identify and characterize the LA type. These tests are reported to be more sensitive, but at present there is no single specific or totally sensitive confirmatory test for a LA. The sensitivity of the APTT test varies depending on the test system used, but most APTT reagents currently available are adequate for diagnosing over 80–90% of patients with inhibitors.[6]

Association with thrombosis

In spite of their *in-vitro* anticoagulant activity, it was soon realized that lupus anticoagulants are only rarely associated with a haemorrhagic diathesis, but paradoxically *in vivo* are associated with thrombosis.[7,8] Boey *et al.* in 1983 reported that the presence of the LA appeared to be a marker for a subset of patients with SLE at risk from thromboembolic episodes,[9] and numerous confirmatory reports have followed. Up to 30% of patients with a LA develop a thrombosis.[8] This most commonly affects the deep leg veins, but may occur at an unusual or serious site. Elias and Eldor, however, showed that in a retrospective study of a group of patients with detectable LAs, more than 50% suffered thrombotic episodes.[10] The thromboses occurred with equal frequency in patients with and without SLE.

Haemorrhagic episodes in patients who have the anticoagulant appear only to occur when a second

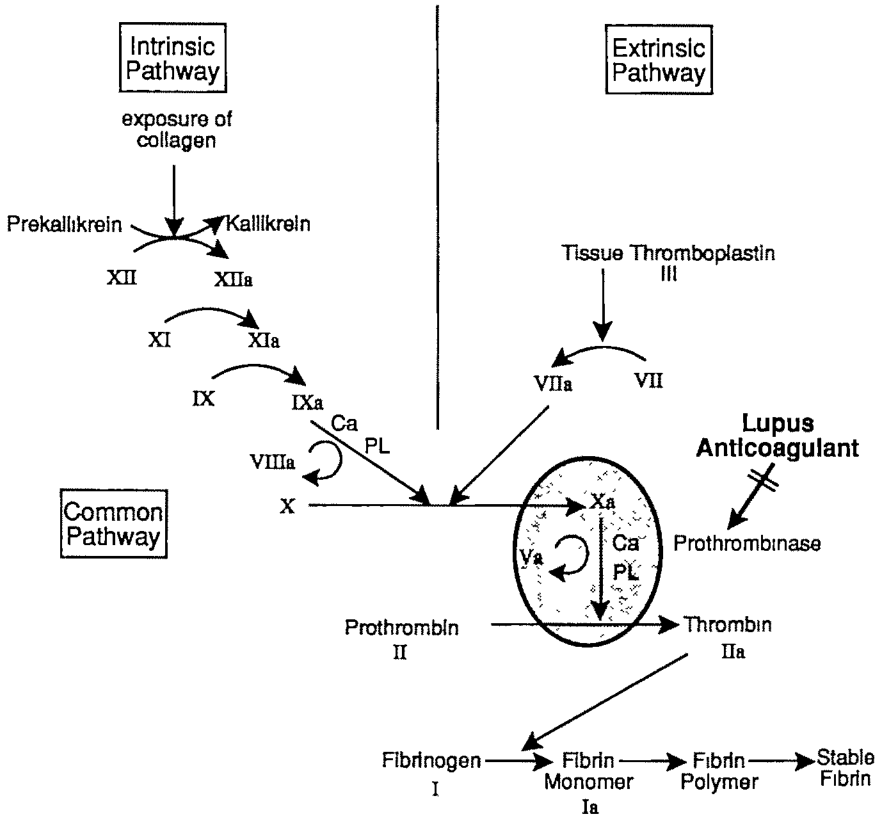

Figure 1. Site of action of the lupus anticoagulant in the clotting cascade

haemostatic defect is present, usually thrombocytopenia.

The addition of phospholipids or platelets to the test systems used to detect the LA may partially or fully correct the defect[7,11] and activated platelets were found to overcome the effect of the anticoagulant in both the intrinsic and extrinsic coagulation pathways.[12] If activated platelets are present at the site of vessel trauma, this may explain why patients with the LA rarely suffer haemorrhagic tendencies despite the profound effect seen on *in-vitro* coagulation tests. Because the clinical manifestation of this antibody is normality or thrombosis, the term 'lupus anticoagulant' is a misnomer.

Anticardiolipin antibodies

The clinical associations of the LA were soon extended to include recurrent foetal loss[13,14] and thrombocytopenia,[15] and these patients were frequently found to have a false positive VDRL reaction. Syphilis antibodies bind to a phospholipid known as cardiolipin, which with phosphatidyl choline and cholesterol make up the VDRL reagent. It was not unreasonable to assume therefore that cardiolipin was the phospholipid against which the LA was directed, and thus cardiolipin was used to devise a radioimmunoassay[16] subsequently modified to an ELISA technique[17] to demonstrate these antibodies in the sera of patients. The advantages offered by solid-phase anticardiolipin immunoassays are that large numbers of sera may be rapidly screened, the results are less affected by sample preparation, transportation, and storage than tests for the LA, the results can be quantified, standardization and quality control should be readily achievable, and results are not affected by concomitant anticoagulant therapy. The one main drawback of the anticardiolipin test is its sensitivity which can give rise to frequent false positive results. Anticardiolipin antibodies are not specific for cardiolipin, but bind to any phospholipid with a negatively charged phosphodiester group (e.g. cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidic acid).[17] Cross-reactivity with positively charged phospholipid seldom occurs. It is thought that the addition of a positively charged group, such as choline as in phosphatidylcholine, causes a charge-to-charge interaction with the negatively charged phosphodiester group so blocking access by antiphospholipid antibodies.[15] DNA comprises a phosphodiester-linked backbone similar to the polar region of cardiolipin, and monoclonal anticardiolipin antibodies raised in mouse–mouse hybridomas have been shown to cross-react with DNA.[19] Cross-reactivity with DNA would explain the wide range of autoantibodies seen in SLE, by an ubiquitous antigen such as negatively charged phospholipid being able to stimulate autoantibodies with apparently different specificities, such as anticardiolipin and anti-DNA antibodies. However, further studies have shown no evidence of cross-reactivity or clinical correlation between these antibodies.[20] Phospholipids associate in aqueous solution in miceller, hexagonal or other configurations, and Janoff and Ranch[21] demonstrated that antiphospholipid antibodies react with positively charged phosphatidylethanolamine in a hexagonal but not lamellar configuration. A significant recent article has shown that a plasma protein cofactor, aca-cofactor, is necessary for binding of anticardiolipin antibodies to cardiolipin liposomes. Aca-cofactor is a 50-kDa plasma peptide which itself has a high affinity for phospholipids, and has properties similar to B2-glycoprotein.[22]

Relationship between anticardiolipin antibodies and lupus anticoagulants

It should be stressed that lupus anticoagulant tests and ELISAs or radioimmunoassays for cardiolipin antibodies are different ways of detecting a very heterogeneous spectrum of antibodies to phospholipids. There is a close but not absolute correlation between the cardiolipin assay and tests for the LA. The majority of cardiolipin-positive patients have a LA but only 60–70% of patients with the LA have detectable cardiolipin antibodies, although all are at risk of thrombosis.[23] Furthermore, there is no relationship between the degree of prolongation of the APTT test and the titre of cardiolipin antibody, and there are reports of patients whose LA has become undetectable after prednisolone therapy when the anticardiolipin titre has remained highly positive.[24] There is also substantial evidence supporting an association between the two parameters: the clinical features associated with a LA and with anticardiolipin antibodies are the same, and both tests are frequently positive (although this does not signify even greater risk of thrombosis). LA activity can be absorbed out by cardiolipin lysosomes.[25] LA tests are most sensitive using low concentrations of phospholipids[26] and can be neutralized by adding phospholipid.[27,28] Furthermore, immunoglobulin isolated from LA-positive patients has recently been shown to bind cardiolipin and negatively charged phospholipid.[25]

Although there are differences in test sensitivity, this does not fully explain the discrepancy between LA activity and anticardiolipin antibodies. It is quite possible that different phospholipids are being detected by the two tests, and that only certain conformational states of phospholipid allow binding of anticardiolipin antibodies. Alternatively, antibodies with specificities other than for phospholipids may give positive LA test results.[29,30] Most investigators agree that both anticardiolipin and lupus anticoagulant tests should be performed in patients suspected of having antiphospholipid antibody-related thrombosis or foetal loss.

Clinical correlations

Anticardiolipin antibodies (aCL) were initially described in SLE, and have been detected in approximately 10–30% of patients with SLE by the ELISA technique.[16,31] All three major immunoglobulin classes have been detected. In one large centre, IgG anticardiolipin antibody appears to correlate best with disease activity, and symptoms occur more frequently as the level of antibody rises.[32] However, these findings have not been confirmed in other large studies.[23] Clinical complications occur at unpredictable times and do not appear to be related to changes in antibody level.

It is well recognized that antiphospholipid antibodies are not confined to patients with SLE. Lupus anticoagulants have also been detected in patients with other autoimmune disorders including mixed connective tissue disease, polyarteritis nodosa, primary sicca syndrome, discoid LE, systemic sclerosis and autoimmune haemolytic anaemia.[9,10,15] They are also reported in Behçets disease,[33] drug-induced LE,[8,34–36] carcinoma,[15] and in some normal people.[37] More recently it has been recognized that a high percentage of patients with AIDS have circulating LAs.[38,39] Thus, the concept of an antiphospholipid syndrome is evolving, encompassing a clinical spectrum that is far wider than just SLE alone.

Clinical features associated with antiphospholipid antibodies: the antiphospholipid syndrome

Recurrent venous thrombosis is associated with the lupus anticoagulant and anticardiolipin antibodies.[8,9,16,40] Thrombosis of leg veins has been most frequently reported and is often accompanied by pulmonary embolism. Secondary pulmonary hypertension also occurs.[41] Other sites affected are renal veins,[42] hepatic veins, associated with a Budd Chiari syndrome,[43] retinal veins,[44] the axillary veins[9] and superficial thrombophlebitis.[45]

Arterial occlusions are common, particularly involving the large cerebral arteries causing a stroke or transient ischaemic attacks (frequently amaurosis fugax).[18,46,47] In a study of 15 lupus patients with cerebral thrombosis, 13 had high levels of aCL antibody. The thromboses were often multiple and catastrophic, and were frequently seen in otherwise quiescent cases of lupus.[46] Other neurological disorders encountered are multi-infarct dementia, epilepsy and chorea.[48] More recently, the association with myocardial infarction, particularly in the young, has been stressed.[49]

In women with or without SLE, the presence of the lupus anticoagulant is associated with a very high incidence of first trimester spontaneous abortions and second and third trimester foetal deaths.[9,10,14,50–53] Several retrospective reviews have shown a foetal survival rate of only 11–14%. More recently it has also been shown that women with SLE and high levels of anticardiolipin antibodies are similarly at risk of recurrent foetal loss, thrombosis or both.[13,54] Extensive placental infarction is commonly seen histologically.[13,50,53] More recently, a distinct post-partum syndrome has been identified in patients with antiphospholipid antibodies consisting of spiking fevers, chest pain and dyspnoea. Chest X-rays showed pleural effusions and patchy infiltrates, and venous thromboses were demonstrated in two patients.[55]

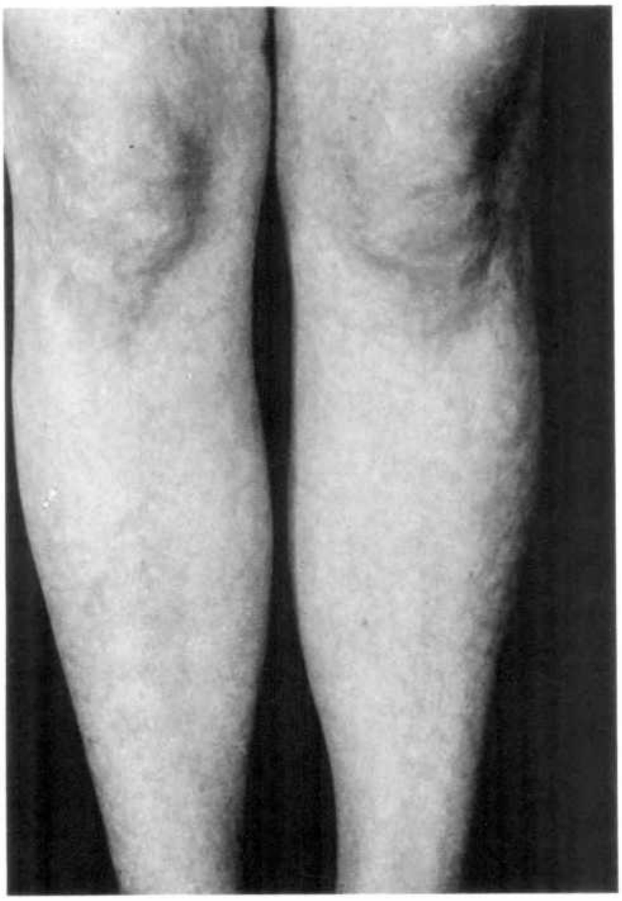

Figure 2. Livedo reticularis in a patient with a lupus anticoagulant.

Thrombocytopenia is a common finding in patients with APL antibodies, but it is not usually associated with haemorrhage. One theory as to its aetiology is that antiphospholipid antibodies may bind phospholipid in platelet membranes resulting in hyperaggregatability causing thrombosis, and enhanced uptake and destruction by the reticuloendothelial system. A frequency of antiphospholipid antibodies of approximately 30% occurs in patients with idiopathic thrombocytopenic purpura.[56]

The term antiphospholipid syndrome has recently been introduced to describe patients with anticardiolipin antibodies, or a lupus anticoagulant and at least one of the following clinical features: venous or arterial thrombosis, recurrent foetal loss or thrombocytopenia. Serological tests must be positive on at least two occasions, more than 3 months apart.[57]

Cutaneous manifestations associated with antiphospholipid antibodies

There are numerous isolated case reports of skin lesions occurring in patients with antiphospholipid antibodies, and several group studies showing an increased frequency of certain clinical signs in these patients. Clinical features include livedo reticularis[58–60] (Fig. 2), leg ulcers[61–63] (Fig. 3), necrotizing purpura,[64,65] Sned-

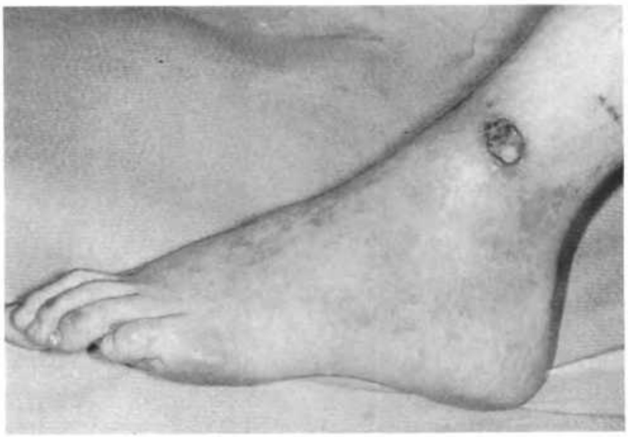

Figure 3. Sharply marginated ulcer, and atrophie-blanche at a site of previous ulceration, in a young woman with cardiolipin antibodies.

don's syndrome (livedo reticularis associated with cerebrovascular disease) with livedo vasculitis,[66] distal cutaneous ischaemia,[67] widespread cutaneous necrosis (Fig. 4),[68–70] peripheral gangrene,[71,72] thrombophlebitis,[45,63] and haemorrhage.[63] Several terms have been used to describe the same clinical entity, for example cutaneous necrosis, necrotizing purpura and cutaneous gangrene, but the histology of most cases reveals changes probably related to the coagulation defect. Dermal vessel thrombosis, usually without vasculitis is seen, and may be considered characteristic of the skin lesions associated with antiphospholipid antibodies (Fig. 5).[73] Dermal thrombosis is not however specific, and identical cutaneous lesions are seen in thrombotic thrombocytopenia purpura, disseminated intravascular coagulation, cryoglobulinaemia, purpura fulminans and warfarin necrosis in protein C deficiency. Cutaneous ulceration is often characteristic and shares many clinical features with livedo vasculitis as described by Bard and Winkelmann.[74] Painful, sharply marginated ulcers are seen, often grouped around the ankles, which heal very slowly to leave scarring and prominent atrophie blanche (Fig. 3). Isolated reports have also raised the possibility of an association between Sneddon's syndrome,[66,75] Behçets disease[33] and malignant atrophic papulosis (Degos disease)[76] and antiphospholipid antibodies, but confirmatory studies are obviously required.

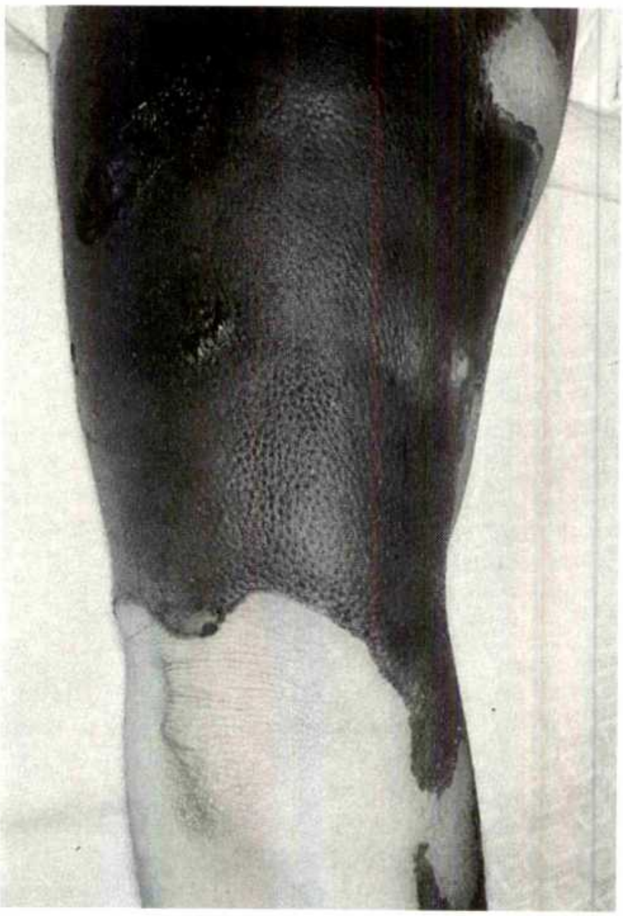

Figure 4. Extensive cutaneous necrosis in a patient with a lupus anticoagulant.

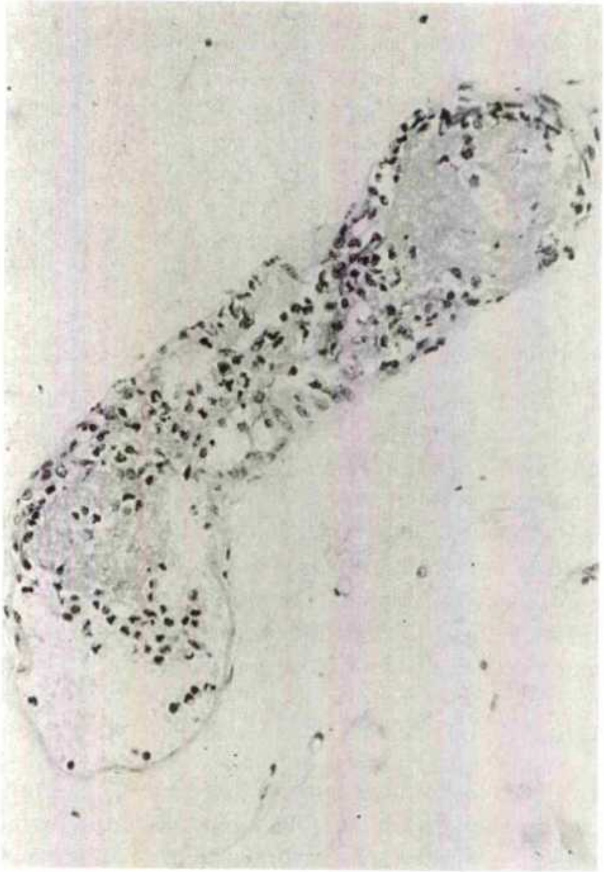

Figure 5. Histology of perilesional skin from the patient in Figure 4 showing intradermal vascular thrombosis (× 25).

It is important to look for antiphospholipid antibodies in all patients with thrombotic skin lesions, as in many cases the skin lesion may be the first sign of the disease. In one study, 40% of those patients with cutaneous lesions had multi-system thrombotic events throughout the course of their disease.[63]

The anticoagulant system

The biochemical mechanism for the thrombosis associated with the LA is unknown, and indeed it is still not clear whether the LA has a pathophysiological role in the development of thrombosis, or whether it is only an epiphenomenon. To appreciate the proposed mechanisms of action, a current knowledge of the natural anticoagulant system is required. Coagulation, once initiated, would proceed unchecked unless a system of inhibition was simultaneously brought into play. The clotting system can be thought of as a dynamic process of continuous clot formation and dissolution, occurring under resting circumstances, with massive amplification occurring after vessel injury. Natural anticoagulant activity occurs through the activation and interaction of three pathways: (1) antithrombin III, (2) protein C and protein S, and (3) the fibrinolytic system.

Antithrombin III

This is a naturally occurring serine protease inhibitor which irreversibly binds activated clotting factor XIIa, XIa, IXa, Xa and II (thrombin).[77] Antithrombin III is the primary anticoagulant mechanism of the body and exerts most of its inhibitory effect on factor Xa. Its speed of action is markedly enhanced by heparin and to a lesser extent heparin sulphate, present on the surface of endothelial cells. Antithrombin III deficiency is the commonest cause of inherited thrombophilia.

Protein C

Protein C has been the subject of much research in the past decade, and it is now known that protein C deficiency contributes to two dramatic clinical entities—purpura fulminans and warfarin skin necrosis. Protein C is a vitamin K-dependent glycoprotein, secreted in an inactive zymogen form. It is converted to its active form when thrombin complexes with thrombomodulin, a high affinity thrombin receptor, found on vascular endothelial cell surfaces, which acts as a cofactor.[78,79] The effect is potentiated by the presence of phospholipids and is greatest in the microvasculature where the ratio of surface area of endothelium to volume of blood is large. Protein S is a cofactor for activated protein C. It complexes with protein C on phospholipid vesicles, and together they inhibit fibrin formation by degrading factor Va and VIIIa, and stimulate fibrinolysis by decreasing the activity of plasminogen activator inhibitor (Fig. 6).

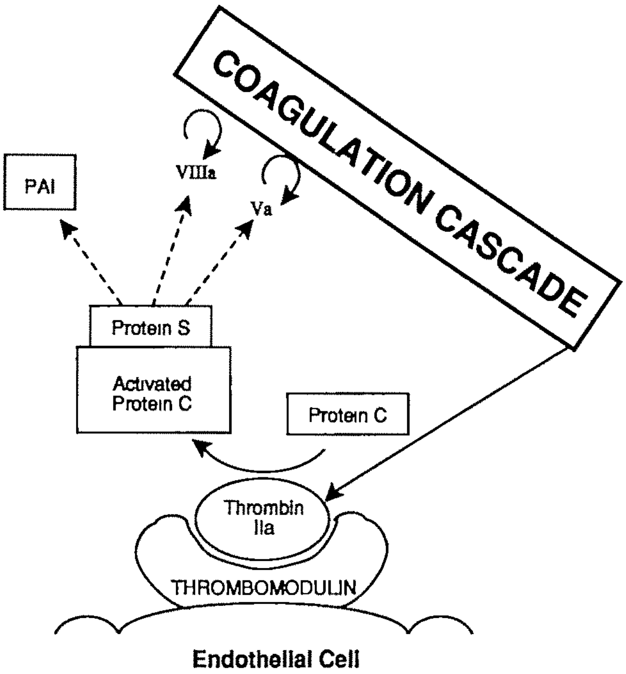

Figure 6. Anticoagulation. Protein C and protein S. PAI, plasminogen activator inhibitor; ——➤, inhibition.

The fibrinolytic system

This involves the conversion of the inactive proenzyme plasminogen to the serine protease plasmin, which is capable of digesting fibrin (Fig. 7).[80] Plasminogen is predominantly synthesized in the liver, and circulates either bound to histidine-rich glycoprotein or in a free form.[81] Fibrinolysis is triggered by three interacting systems: through contact activation of the intrinsic coagulation pathway involving factor XIIa and kallikrein, which simultaneously generates plasmin, and by the enzymatic activities of urokinase and tissue plasminogen activator (tPA). tPA is a protease, synthesized and secreted by endothelial cells,[82] and appears to have the greatest control over fibrinolysis. It binds avidly to fibrin and as a result, plasminogen is maximally activated at the site of thrombus formation, a property that was the primary impetus for its development as a therapeutic agent.[83]

The secretion of tPA by endothelial cells is under the control of a complex series of physical and hormonal factors, being increased by stress, exercise, venous pressure and vasoactive substances, as well as the major enzyme of the coagulation cascade itself, thrombin.[84] Several plasma factors directly inhibit the action of tPA on plasminogen, the most important being α2-antiplasmin and the specific tPA inhibitor, PAI 1, which is synthesized by endothelial cells and platelets.[84] Thrombin also stimulates the secretion of PAI 1,[85] and thus by itself is responsible for the fine balance between coagulation, and the initiation and cessation of clot lysis through the tPA system.

Defective fibrinolysis, leading to thrombophilia, can occur through congenital deficiency or defective release of tPA, or increased PAI activity. There are many reports describing elevated inhibitor levels or defective tPA release in patients with venous thromboses,[40,86] in

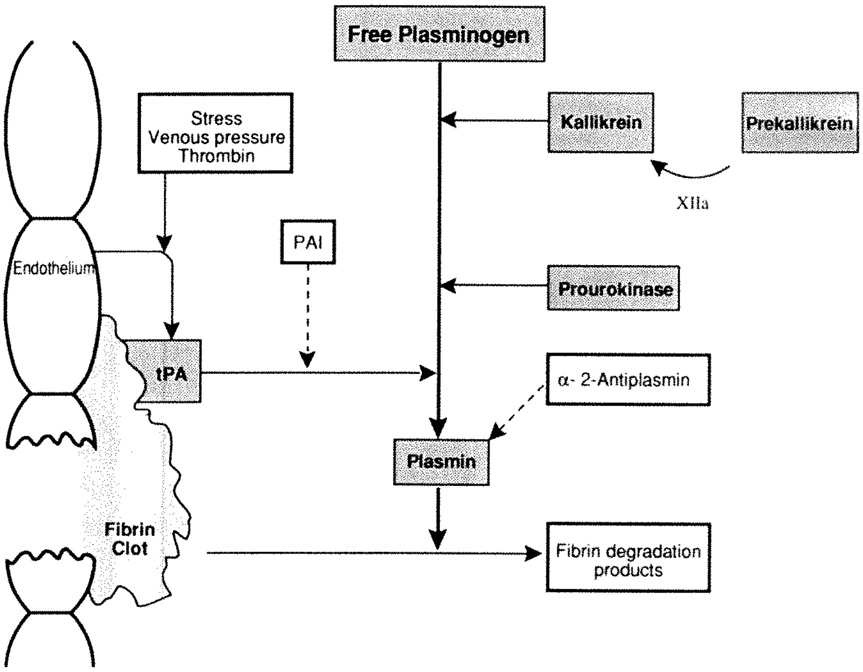

Figure 7. Fibrinolysis. tPA, tissue plasminogen activator, PAI, plasminogen activator inhibitor, — — ➤, inhibition.

cigarette smokers in gram-negative sepsis,[87] and in coronary artery disease.[88] However, at present, fibrinolytic screening tests are difficult to interpret as so many physical factors affect the circulatory level of tPA, and the range of values is wide. The significance of abnormal tPA results must be interpreted with caution.

Mechanism of thrombosis associated with antiphospholipid antibodies

Several mechanisms for thrombosis occurring with antiphospholipid antibodies have been proposed, focusing primarily on dysfunction of the endothelium. Inhibition of prostacyclin release from blood vessels has been demonstrated,[52,89] and it is suggested that this could result from antiphospholipid antibody binding impairing the release of arachidonic acid from phospholipids in the endothelial cell membrane. Because prostacyclin is a potent vasodilator and inhibitor of platelet aggregation, it is regarded as an important defence mechanism against thrombosis, and its impaired synthesis or release might contribute to the thrombotic risk in these patients. However, no such abnormality has been found in similar subsequent studies.[10,90–93] The two largest studies[92,93] evaluated the effect of serum on prostaglandin release from cultured human endothelial monolayers using a standardized assay system for the prostacyclin metabolite 6-keto-prostaglandin F1a. No impairment of agonist-stimulated or mechanically damaged endothelial prostaglandin release was demonstrated by sera containing antiphospholipid antibodies. However Dudley *et al.*[92] showed that the baseline concentration of 6-keto-prostaglandin F1a was nearly four-fold higher in sera containing antiphospholipid antibodies, possibly suggesting a state of long-term stimulation or altered metabolism of prostaglandin. It is of interest that those studies based on the use of plasma generally show inhibition of prostaglandin release, whereas reports based on the use of serum fail to do so, possibly due to the release of masking stimulatory factors by platelets during coagulation.

Other investigators have demonstrated reduced activation of protein C by interaction of LA with endothelial bound thrombomodulin[94–96] but this would not satisfactorily explain arterial thrombosis which does not occur in hereditary protein C deficiency. Interference with antithrombin III activity[97] is reported, and inhibition of prekallikrein was demonstrated in three patients with the LA as compared with normal plasma.[98] Several reports have demonstrated a decreased tissue plasminogen activator release in response to venous occlusion in patients with the lupus anticoagulant[99–101] and others have shown significantly elevated plasminogen activator inhibitor levels[102,103] but could demonstrate no correlation with thrombosis. In addition, it has been demonstrated that there was increased von Willebrand factor activity in patients with SLE,[100,101] but there was no correlation between either finding and thrombotic events. More recently, however, elevated levels of tPA and PAI have been demonstrated in patients with SLE complicated by a history of thrombosis or recurrent abortions.[104]

Therapy

The treatment and prevention of thrombosis in patients with LA/aCL antibodies is still largely experimental, and many large-scale prospective studies are needed in order to determine the most effective and least harmful therapy. Treatment has been aimed at reducing the antibody levels by immunosuppression and plasmaphoresis, and favourable reports have appeared on the use of systemic steroid therapy in antiphospholipid antibody-positive women with recurrent spontaneous abortions and intrauterine death.[53,105] Lubbe *et al.* reported six women with SLE and recurrent intrauterine deaths, all of whom had the lupus anticoagulant.[53] Treatment with prednisolone 50 mg/day and low-dose aspirin resulted in lowering of LA activity and a successful pregnancy outcome in five.[53] Successful pregnancies in five out of eight women with the LA, but not SLE were also achieved,[51] reducing the rate of pregnancy loss from 96·8 to 37·5%. The foetal outcome was not related to normalization of the coagulation tests. However, pre-eclampsia occurred in all five women who gave birth to live infants, and foetal growth retardation in three cases.[51] Meticulous antenatal care, preferably in a specialist centre, for all aCL positive women is important. Foetal heart-rate monitoring should be undertaken from the earliest time the foetus is viable, and if progressive deterioration occurs with cardiac decelerations, delivery should be undertaken.[54]

Another report, one of widespread cutaneous necrosis associated with the LA showed the process to be halted by prednisolone when heparin had been ineffective.[68] Two other reports showed disappearance of the anticoagulant after high-dose corticosteroid therapy[2,106] although it was found that the use of corticosteroids, NSAIDs, including aspirin, immunosuppressive and cytotoxic agents had no effect on the presence of the LA even when given for long periods of time.[10]

The alternative management approach is to prevent the thrombotic effects of antiphospholipid antibodies. Warfarin therapy has been recommended for recurrent thrombotic episodes, and there is good evidence that this treatment is effective. Continuous therapy appears to prevent clotting and recurrent thrombosis on warfarin withdrawal is well documented.[10,107] It is also suggested that anticoagulation should be kept in the high therapeutic range. However, the risks of committing a patient to life-long warfarin administration are significant, and have to be weighed against the risks of serious thrombosis.

Other antithrombotic agents may also be useful, particularly for arterial occlusive events. Two patients with TIAs and a LA responded well to aspirin 0·3 g/day with dipyridamole 225 mg/day.[10] The combination of aspirin and dipyridamole has also been shown to be of benefit in patients with atrophie blanche or livedo vasculitis of the lower limb manifesting clinically as chronic painful ulceration.[108,109] The nine patients studied all had abnormal platelet function, but in all, there was significant alleviation of pain and decrease in new lesion formation with treatment. Furthermore, aspirin and dipyridamole caused healing where prednisolone had failed in a case of livedo vasculitis associated with Sneddon's syndrome and anticardiolipin antibodies.[66] Aspirin is a cyclo-oxygenase inhibitor that acts on both platelets and vascular endothelial cells to suppress synthesis of thromboxane A2 and prostaglandin 12 respectively. Thus, thrombus formation and platelet aggregation are both suppressed. The mechanism of action of dipyridamole as an antiplatelet agent however remains unclear. It has been suggested that dipyridamole (i) inhibits the phosphodiesterase enzyme in platelets, resulting in an increase in intraplatelet cyclic AMP; (ii) directly stimulates the release of prostaglandin 12 from vascular endothelium; or (iii) inhibits the uptake and metabolism of adenosine. However, dipyridamole has not in fact been proven to have an antithrombotic effect in humans. In the majority of clinical trials designed to assess the efficacy of dipyridamole, it has been combined with aspirin rather than compared directly with placebo therapy, and therefore it cannot be said to confer additional benefit to aspirin alone.[110]

Currently of interest is the use of specific fibrinolytic drugs at the time of acute thrombosis. It is now well accepted that thrombolytic therapy begun soon after an acute myocardial infarction reduces mortality and preserves left ventricular function. Both intravenous streptokinase[111] and recombinant tissue plasminogen activator[112] have been evaluated in large, randomized clinical trials, and in view of the recent reports of abnormal fibrinolytic activity in patients with aCL antibodies, this therapeutic avenue needs to be explored.

Adrenaline, histamine, nicotinic acid and vasopressin all stimulate fibrinolysis but have a very short duration of action. Phenformin and the anabolic steroid stanozolol also produce fibrinolytic enhancement alone or in combination, in both healthy adults and in patients with various forms of vascular disease associated with fibrinolytic impairment.[113–115] Lactic acidosis, the rare but life-threatening side-effect of phenformin, now precludes the use of this drug, but stanozolol therapy has been used successfully in a number of clinical trials to treat patients with cutaneous vasculitis,[116] Raynaud's phenomenon,[117] venous lipodermatosclerosis,[118] and recurrent thrombophlebitis.[119] Stanozolol increases plasminogen levels, increases plasminogen activator levels, and lowers the levels of the plasmin inhibitor α2-macroglobulin and of fibrinogen. It has also recently been shown to lower levels of plasminogen activator inhibitor,[120] and therefore would seem a possible therapeutic option for patients with antiphospholipid antibodies in spite of the mild masculinizing effects that occur in some women. Finally, vasodilator drugs may also help prevent clot formation by increasing the rate of blood flow through the microvasculature, thereby preventing the initiation of fibrin deposition. In this respect, nifedipine has been used to treat idiopathic atrophie blanche in a case which failed to respond to aspirin and dipyridamole.[121] Nifedipine is also anecdotally reported to have fibrinolytic activity,[122] and indeed has been shown to reverse PAI abnormalities in a patient with widespread cutaneous necrosis associated with the lupus anticoagulant.[123]

Treatment guidelines for patients with antiphospholipid antibodies

Although there is a lack of controlled prospective trials, in the light of available knowledge, the following treatment is recommended.

1. Asymptomatic patients with the LA or aCL antibodies detected as an incidental finding: low-dose aspirin (75–150 mg) daily, lifelong.
2. Patients with a history of a single DVT: short-term anticoagulation at times of high risk of thrombosis (e.g. surgery). Self-administered subcutaneous heparin should be used throughout the risk period, and warfarin taken for 3–4 months afterwards.
3. Patients with multiple DVTs or a serious thrombotic event: long-term low-dose aspirin and warfarin, with anticoagulation kept in the high therapeutic range.
4. Patients with arterial occlusions: long-term low-dose aspirin.
5. Pregnancy: patients with lupus should be advised to wait until their disease is in remission before conception. Scrupulous antenatal care, preferably in a specialist centre, is of great importance. Patients with no thrombotic history should be put on low-dose aspirin. Patients with a history of thrombosis require anticoagulation throughout pregnancy in addition to aspirin. Subcutaneous heparin is necessary for the early stages of pregnancy, but should not be used for more than a few months because of its osteoporotic effect. Therefore, women should remain on warfarin, and as soon as they become pregnant convert to heparin, and then return to warfarin in late pregnancy. At present, there is insufficient evidence of additional benefit from adding corticosteroids in pregnancy.

References

1 Conley CL, Hartmann RC. A haemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J Clin Invest* 1952; **31:** 621–2.

2 Feinstein DI, Rapaport SI. Acquired inhibitors of blood coagulation. In: *Progress in Haemostasis and Thrombosis* (Spaet T.H., ed.), Vol 1. New York: Grune and Stratton, 1972; 75–95.

3 Shapiro S, Thiagarajan P. Lupus anticoagulant. *Prog Haemost Thromb* 1982; **6:** 263–85.

4 Thiagarajan P, Pengo V, Shapiro SS. The use of dilute Russel viper venom time for the diagnosis of lupus anticoagulants. *Blood* 1986; **63:** 869–74.

5 Exner T, Rickard KA, Kronenberg H. A sensitive test demonstrating the lupus anticoagulant and its behavioural patterns. *Br J Haematol* 1978; **40:** 143–51.

6 Green D, Hougie C *et al.* Report of the working party on acquired inhibitors of coagulation: Studies of the lupus anticoagulant. *Thromb Haemostas* 1983; **49:** 144–6.

7 Bowie EJ, Thompson JH, Pascuzzi CA, Owen CA. Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. *J Lab Chin Med* 1963; **63:** 416–30.

8 Mueh JR, Herbst KD, Rapaport SI. Thrombosis in patients with the lupus anticoagulant. *Ann Intern Med* 1980: **92:** 156–9.

9 Boey ML, Coalaco CB, Gharavi AE *et al.* Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. *Br Med J* 1983: **287:** 1021–3.

10 Elias M, Eldor A. Thromboembolism in patients with the 'lupus' type circulating anticoagulant. *Arch Intern Med* 1984; **144:** 510–15.

11 Exner T, Rickard KA, Kronenberg H. Studies on Phospholipids in the action of a lupus coagulation inhibitor. *Pathology* 1975; **7:** 319–28.

12 Firkin BG, Booth P, Hendrix L, Howard MA. Demonstration of a platelet bypass mechanism in the clotting system using an acquired anticoagulant. *Am J Haematol* 1976; **5:** 81–92.

13 Derue GJ, Englert HJ, Harris EN *et al.* Fetal loss in systemic lupus erythematosus: association with anticardiolipin antibodies. *J Obst Gynaecol* 1985; **5:** 207–9.

14 Nilsson IM, Astedt B, Hedner V, Berezin D. Intrauterine death and circulating anticoagulant (antithromboplastin). *Acta Med Scand* 1975; **197:** 153–9.

15 Schleider MA, Nachmann RL, Jaffe EA, Coleman M. A clinical study of the lupus anticoagulant. *Blood* 1976; **48:** 499–509.

16 Harris EN, Gharavi AE, Boey ML *et al.* Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983; **ii:** 1211–14.

17 Gharavi AE, Harris EN, Asherson RA, Hughes GRV. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. *Ann Rheum Dis* 1987; **46:** 1–6.

18 Hughes GRV. Thrombosis, abortion, cerebral disease and the lupus anticoagulant. *Br Med J* 1983; 287: 1088–9.

19 Rauch J, Tannenbaum H, Stollar BD, Schwartz RS. Monoclonal anticardiolipin antibodies bind DNA. *Eur J Immunol* 1984; 14: 529–34.

20 Harris EN, Gharavi AE, Tincani A *et al.* Affinity purified anticardiolipin and anti-DNA antibodies. *J Clin Lab Immunol* 1985; 17: 155–62.

21 Janoff AS, Rauch J. The structural specificity of antiphospholipid antibodies in autoimmune disease. *Chem Physics Lipids* 1986; 40: 315–22.

22 Galli M, Comfurius P, Maassen C *et al.* Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 1990; 335: 1544–7.

23 Triplett DA, Brandt JT, Musgrave KA, Orr CA. Relationship between lupus anticoagulants and antibodies to phospholipids. *J Am Med Assoc* 1988; 259: 550–4.

24 Derksen RHWM, Biesma D, Bouma BN. Discordant effects of prednisolone on anti-cardiolipin antibodies and the lupus anticoagulant. *Arth Rheumat* 1986; 29: 1295–6.

25 Pengo V, Heine MJ, Thiagarajan P, Shapiro SS. Immunological specificity and mechanism of action of IgG lupus anticoagulants. *Blood* 1987; 70: 69–76.

26 Exner T, Rickard KA, Kronenberg H. Studies on phospholipids in the action of lupus anticoagulant inhibitor. *Pathology* 1975; 7: 319–28

27 Firkin BG, Booth P, Hendrix I, Howard MA. Demonstration of a platelet bypass mechanism in the clotting system using an acquired anticoagulant. *Am J Haematol* 1978; 5: 81–92.

28 Triplett DA, Brandt JT, Kaczor D, Schaeffer J. Laboratory diagnosis of lupus inhibitors: a comparison of the tissue thromboplastin inhibition procedure with a new platelet neutralization procedure. *Am J Clin Pathol* 1983; 79: 678–82.

29 Fleck RA, Rapaport SE, Rao LVM. Anti-prothrombin antibodies and the lupus anticoagulant. *Blood* 1988; 72: 512–19.

30 Exner T, Sahman N, Trudinger B. Separation of anticardiolipin antibodies from lupus anticoagulation on a phospholipid coated polystyrene column. *Biochem Biophys Res Comm* 1988; 155: 1001–7.

31 Harris EN, Gharavi AE, Hughes GRV. Antiphospholipid antibodies. *Clin Rheum Dis* 1985; 11: 591–609.

32 Hughes GRV, Harris EW, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol* 1986; 13: 486–9.

33 Hull RG, Harris EN, Gharavi AE *et al.* Anticardiolipin antibodies: occurrence in Behcet's syndrome. *Ann Rheum Dis* 1984; 43: 746–8.

34 Bell WR, Boss GR, Wolfson JS. Circulating anticoagulant in the procainamide-induced lupus syndrome. *Arch Intern Med* 1977; 137: 1471–3.

35 Canoso RT, Sise HS. Chlorpromazine-induced lupus anticoagulant and associated immunologic abnormalities. *Am J Haematol* 1982; 13: 121–9.

36 Zarrabi MH, Zucker S, Miller F *et al.* Immunologic and coagulation disorders in chlorpromazine-treated patients. *Ann Intern Med* 1979; 91: 194–9.

37 Duran-Suarez JR. Incidence of circulating anticoagulants in normal population. *Acta Haematol (Basel)* 1982; 67: 217–9.

38 Bloom EJ, Abrams DI, Rodgers G. Lupus anticoagulant in the acquired immunodeficiency syndrome. *J Am Med Assoc* 1986: 256: 491–3.

39 Cohen AJ, Philips TM, Kessler CM Circulating coagulation inhibitors in the acquired immunodeficiency syndrome. *Ann Int Med* 1986; 104: 175–80.

40 Jorgensen M, Mortensen JZ, Madsen AG, *et al.* A family with reduced plasminogen activator activity in blood, associated with recurrent venous thrombosis. *Scand J Haematol* 1982; 29: 217–23.

41 Asherson RA, Mackworth-Young CG, Boey ML *et al.* Pulmonary hypertension in systemic lupus erythematosus. *Br Med J* 1983. 287: 1024–5.

42 Asherson RA, Lanham JG, Hull RG *et al.* Renal vein thrombosis in systemic lupus erythematosus; association with the 'lupus anticoagulant' *Clin Exp Rheumatol* 1984; 2: 75–9.

43 Farrant JM, Judge M, Thompson RPH. Thrombotic cutaneous nodules and hepatic vein thrombosis in the anticardiolipin syndrome. *Clin Exp Dermatol* 1989; 14: 306–8.

44 Hall S, Buettner H, Harrinder LS. Occlusive retinal vascular disease in systemic lupus erythematosus. *J Rheumatol* 1984; 11: 846–50.

45 Peck B, Hoffman GS, Franck WA. Thrombophlebitis in systemic lupus erythematosus. *J Am Med Assoc* 1978; 240: 1728.

46 Harris EN, Gharavi AE, Asherson RA *et al.* Cerebral infarction in systemic lupus: Association with anticardiolipin antibodies. *Clin Exp Rheumatol* 1984; 2: 47–51.

47 Harris EN, Hughes GRV. Cerebral disease in systemic lupus erythematosus. Springer, *Seminars in Immunopathology* 1985, 8: 251–66.

48 Asherson RA, Derksen RHWM, Harris EN *et al.* Chorea in systemic lupus and lupus-like disease. *Sem Arthr Rheumatol* 1986.

49 Hamsten A, Norberg R, Bjorkholm M *et al.* Antibodies to cardiolipin in young survivors of myocardial infarction: an association with recurrent cardiovascular events. *Lancet* 1986; i: 113–15.

50 De Wolf F, Carreras LO, Hoerman P *et al.* Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss and a lupus anticoagulant. *Am J Obst Gynaecol* 1982; 142: 829–34.

51 Ware-Branch D, Scott JT, Kochenour NK, Hershgold E. Obstetric complications associated with the lupus anticoagulant. *New Engl J Med* 1985; 313: 1322–6.

52 Carreras LO, Defreyn G, Machin SJ *et al.* Arterial thrombosis, intrauterine death and 'lupus' anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. *Lancet* 1981; i: 244–6.

53 Lubbe WF, Palmer SJ, Butler WS *et al.* Fetal survival after Prednisolone suppression of maternal lupus anticoagulant. *Lancet* 1983; i: 1361–3.

54 Lockshin MD, Druzin ML, Goei S *et al.* Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. *N Engl J Med* 1985; 313: 152–6.

55 Ware-Branch D, Kochenour NK, Rote NS, Scott JR. Post-partum syndrome associated with antiphospholipid antibodies *Proceedings of 2nd International Symposium on Anticardiolipin Antibodies*, 1986.

56 Harris EN, Gharavi AE, Hedge V *et al.* Anticardiolipin antibodies in the autoimmune thrombocytopenic purpura. *Br J Haematol* 1985; 59: 231–4.

57 Harris EN. Antiphospholipid antibodies. *Br J Haematol* 1990; 74: 1–9.

58 Hughes GRV. Connective tissue disease and the skin. *Clin Exp Dermatol* 1984; 9: 535–44.

59 Asherson RA, Mayou SC, Merry P *et al.* The spectrum of livido reticularis and anticardiolipin antibodies. *Br J Dermatol* 1989; 120: 215–21.

60 Weinstein C, Miller MH, Axtens R *et al.* Livido reticularis associated with increased titers of anticardiolipin antibodies in

systemic lupus erythematosus. *Arch Dermatol* 1987; **123:** 590–600.

61 Cuny JF, Schmutz JL, Jeandel C *et al.* Ulcere de jambe et anticoagulant circulants. *Ann Dermatol Venereol* 1986; **113:** 825–6.

62 Grob JJ, Bonerandi JJ. Cutaneous manifestations associated with the presence of the lupus anticoagulant: a report of two cases and a review of the literature. *J Am Acad Dermatol* 1986; **15:** 211–19.

63 Alegre VA, Gastineau DA, Winkelmann RK. Skin lesions associated with circulating lupus anticoagulant. *Br J Dermatol* 1989; **120:** 419–29.

64 Johansson EA, Niemi KM, Mustakallio KK. A peripheral vascular syndrome overlapping with systemic lupus erythematosus. *Dermatologica* 1977; **155:** 257–67.

65 Naldi L, Marchesi L, Finazzi G *et al.* Antiphospholipid antibodies and necrotizing purpura. *Dermatologica* 1990; **180:** 272–5.

66 Grattan CEH, Burton JL, Boon AP. Sneddon's syndrome (livedo reticularis and cerebral thrombosis) with livedo vasculitis and anticardiolipin antibodies. *Br J Dermatol* 1989; **120:** 441–7.

67 Bird AG, Lendrum R, Asherson RA *et al.* Disseminated intravascular coagulation, antiphospholipid antibodies and ischaemic necrosis of extremities. *Ann Rheum Dis* 1987; **46:** 251–5.

68 Dodd HJ, Sarkany I, O'Shaughnessy D. Widespread cutaneous necrosis associated with the lupus anticoagulant. *Clin Exp Dermatol* 1985; **10:** 581–6.

69 Frances C, Tribout B, Boisnic S *et al.* Cutaneous necrosis associated with the lupus anticoagulant. *Dermatologica* 1989; **178:** 194–201.

70 O'Neill A, Gatenby PA, McGaw B *et al.* Widespread cutaneous necrosis associated with cardiolipin antibodies. *J Am Acad Dermatol* 1990; **22:** 356–9.

71 Asherson RA, Derkson RHWM, Harris EN *et al.* Large vessel occlusion and gangrene in systemic lupus erythematosus and 'Lupus-like' disease: a report of 6 cases. *J Rheumatol* 1986; **13:** 740–7.

72 Jindal BK, Martin MF, Gayner A. Gangrene developing after minor surgery in a patient with undiagnosed systemic lupus erythematosus and lupus anticoagulant. *Ann Rheum Dis* 1983; **42:** 347–9.

73 Alegre VA, Winkelmann RK. Histopathologic and immunofluorescence study of skin lesions associated with circulating lupus anticoagulant. *J Am Acad Dermatol* 1988; **19:** 117–24.

74 Bard JW, Winkelmann RK. Livedo vasculitis: segmental hyalinizing vasculitis of the dermis. *Arch Dermatol* 1967; **96:** 489–99.

75 Jonas J, Kolbe K, Volcker HE *et al.* Central retinal artery occlusion in Sneddon's disease associated with antiphospholipid antibodies. *Am J Ophthalmol* 1986; **102:** 37–40.

76 Englert HJ, Hawkes CH, Boey ML *et al.* Degos disease: association with anticardiolipin antibodies and the lupus anticoagulant. *Br Med J (Clin Res)* 1984; **289:** 576.

77 High KA. Antithrombin III, protein C, and protein S. Naturally occurring anticoagulant proteins. *Arch Pathol Lab Med* 1988; **112:** 28–36.

78 Clouse LH, Comp PC. The regulation of haemostasis: the protein C system. *N Engl J Med* 1986; **314:** 1296–304.

79 Miller SJ. The dermatologist and protein C. *J Am Acad Dermatol* 1988; **19:** 904–7.

80 Miyashita C, Wenzel E, Heiden M. Plasminogen: a brief introduction into its biochemistry and function. *Haemostasis* 1988; **18** (Suppl.): 7–13.

81 Takada A, Takada Y. Physiology of plasminogen: with special reference to activation and degradation. *Haemostasis* 1988; **18** (Suppl.): 25–35.

82 Levin EG. Latent tissue plasminogen activator produced by human endothelial cells in culture: evidence for an enzyme-inhibitor complex. *Proc Natl Acad Sci USA* 1983; **80:** 6804–8.

83 Runge MS, Quertermous T, Haber E. Plasminogen activators. The old and the new. *Circulation* 1989; **79:** 217–24.

84 Loscalzo J, Braunwald E. Tissue plasminogen activator. *N Engl J Med* 1988; **319:** 925–31.

85 Gelehrter TD, Snyder-Laszuk R. Thrombin induction of plasminogen activator-inhibitor in cultured human endothelial cells. *J Clin Invest* 1986; **77:** 165–9.

86 Stead NW, Bauer KA, Kinney TR *et al.* Venous thrombosis in a family with defective release of vascular plasminogen activator and elevated plasma factor VIII/von Willebrand's factor. *Am J Med* 1983; **74:** 33–9.

87 Suffredini AF, Harpel PC, Parrilo JE. Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. *N Engl J Med* 1989; **320:** 1165–72.

88 Olofsson BO, Dahlen G, Nilsson TK. Evidence for increased levels of plasminogen activator inhibitor and tissue plasminogen activator in plasma of patients with angiographically verified coronary heart disease. *Eur Heart J* 1989; **10:** 77–82.

89 Carreras LO, Vermylen JG. 'Lupus' anticoagulant and thrombosis—possible role of inhibition of prostacyclin formation. *Thromb Haemostasis* 1982; **48:** 38–40.

90 Rustin MHA, Bull HA, Machin SJ, *et al.* Effects of the lupus anticoagulant in patients with systemic lupus erythematosus or endothelial cell prostacyclin release and procoagulant activity. *J Invest Dermatol* 1988; **90:** 744–8.

91 Hasselaar P, Perkson R, Blokzyl L, De Groot PG. Thrombosis associated with antiphospholipid antibodies cannot be explained by effects on endothelial and platelet prostanoid synthesis. *Thromb Haemostas* 1988; **59:** 80–5.

92 Dudley DJ, Mitchell MD, Ware Branch D. Pathophysiology of antiphospholipid antibodies: absence of prostoglandin-mediated effects on cultured endothelium. *Am J Obstet Gynecol* 1990; **162:** 953–9.

93 Coade SB, van Haaren E, Loizou S *et al.* Endothelial prostacyclin release in systemic lupus erythematosus. *Thromb Haemostas* 1989; **61:** 97–100.

94 Comp PC, De Bault LE, Esmon NE, Esmon CP. Human thrombomodulin is inhibited by IgG from two patients with non-specific anticoagulants. *Blood* 1983; **62:** 1099.

95 Cariou R, Tobelem G, Soria C, Caen J. Inhibition of protein C activation by endothelial cells in the presence of lupus anticoagulant. *N Engl J Med* 1986; **314:** 1193–4.

96 Freyssinet JM, Wiesel ML, Gauchy J *et al.* An IgM lupus anticoagulant that neutralizes the enhancing effect of phospholipid on purified endothelial thrombomodulin activity—A mechanism for thrombosis. *Thromb Haemostas* 1986; **55:** 309–13.

97 Cosgriff TM, Martin BA. Low functional and high antigenic antithrombin III level in a patient with the lupus anticoagulant and recurrent thrombosis. *Arthritis Rheum* 1987; **24:** 94–5.

98 Sanfelippo MJ, Drayna CJ. Prekallikrein inhibition associated with the lupus anticoagulant. *Ann J Clin Pathol* 1982; **77:** 275–9.

99 Chu BP, Russel NH, Powell RJ *et al.* Abnormal fibrinolytic activity in systemic lupus erythematosus and possible mechanisms. *Br J Rheumat* 1988; **27:** 436–9.

100 Angles-Cano E, Sultan Y, Clauvel JP. Predisposing factors to thrombosis in systemic lupus erythematosus. *J Lab Clin Med* 1979; **94:** 312–23.

101 Byron MA, Allington MJ, Chapel HM *et al.* Indications of vascular endothelial cell dysfunction in systemic lupus erythematosus. *Ann Rheumat Dis* 1987; **46:** 741–5.

102 Tsakiris DA, Marbet GA, Makris PE, *et al.* Impaired fibrinolysis as an assential contribution to thrombosis in patients with lupus anticoagulant. *Thromb Haemostas* 1989; **61**: 175–7.

103 Francis RB, McGehee WG, Feinstein DI Endothelial-dependant fibrinolysis in subjects with the lupus anticoagulant and thrombosis *Thromb Haemostas* 1988; **59**: 412–4.

104 Violi F, Ferro D, Valesini G *et al.* Tissue plasminogen activator inhibitor in patients with systemic lupus erythematosus and thrombosis. *Br Med J* 1990; **300**: 1099–102.

105 Farquharson RG, Pearson JF, John L. Lupus anticoagulant and pregnancy management. *Lancet* 1984; **ii**: 228–9.

106 Medal LS, Lisker R. Circulating anticoagulant in disseminated lupus erythematosus. *Br J Haematol* 1959; **5**: 284–93.

107 Asherson RA, Chan JKH, Harris EN *et al.* Anticardiolipin antibody recurrent thrombosis and warfarin withdrawal. *Ann Rheum Dis* 1985; **44**: 823–5.

108 Drucker CR, Duncan WC. Antiplatelet therapy in atrophie blanche and livido vasculitis. *J Am Acad Dermatol* 1982; **7**: 359–63.

109 Yamamoto M, Danno K, Shio H, Imamura S Antithrombotic treatment in livedo vasculitis. *J Am Acad Dermatol* 1988, **18**: 57–62.

110 FitzGerald GA. Dipyridamole-drug therapy. *N Engl J Med* 1987; **316**: 1247–57.

111 ISIS-2. (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both or neither among 17,187 cases of suspected acute myocardial infarction. ISIS-2 *Lancet* 1988; **2**: 349–60.

112 Van de Werf F. Lessons from the European cooperative recombinant tissue plasminogen activator (rt-PA) versus placebo trial. *J Am Coll Cardiol* 1988; 12 (Suppl. A): 14A–19A.

113 Preston FE, Burakowski BK, Porter NR, Malia RG. The fibrinolytic response to stanozolol in normal subjects. *Thromb Res* 1981; **22**: 543–51.

114 Davidson JF, Lockhead M, McDonald GA, McNicol GP. Fibrinolytic enhancement by stanozolol: a double-blind trial. *Br J Haematol* 1972; **22**: 543–59.

115 Menon IS *et al.* Phenformin and stanozolol in blood fibrinolytic activity *Br Med J* 1970; **1**: 428–30.

116 Cunliffe WJ, Dodman B, Roberts BE, Tebbs EM Clinical and laboratory double-blind investigation of fibrinolytic therapy in cutaneous vasculitis. In: *Progress in Chemical Fibrinolysis and Thrombolysis* (Davidson JF, Samama MM, Desnoyers PC, eds). New York: Raven Press, 1975; 1: 323–32.

117 Jarrett PEM, Morland M, Browse N L. Treatment of Raynaud's phenomenon by fibrinolytic enhancement. *Br Med J* 1978, **2**: 523–5.

118 Burnand K, Clemenson G, Morland M *et al.* Venous lipodermatosclerosis· treatment by fibrinolytic enhancement and elastic compression. *Br Med J* 1980; **280**: 7–11.

119 Jarrett PEM, Morland M, Browse NL. Idiopathic recurrent superficial thrombophlebitis. treatment with fibrinolytic enhancement. *Br Med J* 1977; **1**: 933–4.

120 Sue-Ling HM, Davies JA, Prentice CRM *et al.* Effects of oral stanozolol used in the prevention of postoperative deep vein thrombosis on fibrinolytic activity. *Thromb Haemost* 1985; **53**: 141–2.

121 Purcell SM, Hayes TJ. Nifedipine treatment of idiopathic atrophie blanche. *J Am Acad Dermatol* 1986; **14**: 851–4.

122 Rademaker M, Meyrick Thomas RH, Kirby JD, Kovacs IB. Effect of nifedipine on platelets and thrombolysis in systemic sclerosis. *Br J Dermatol* 1988; 119 (Suppl. 33). 25–6.

123 Stephens CJM, Hughes GRV, Savidge GF. Reversal of plasminogen activator inhibitor abnormality by nifedipine in a patient with widespread cutaneous necrosis associated with a lupus anticoagulant 1991 (in press).

British Journal of Dermatology (1991) **125**, 211–216.　ADONIS 000709639100203D

Overexpression of extracellular matrix receptors (VLA-3, 5 and 6) on psoriatic keratinocytes

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Accepted for publication 27 February 1991

Summary The potential role of adhesion molecules in the pathophysiology of psoriasis was investigated and the pattern of expression of the cell-surface receptors ICAM-1, LFA-3, and VLA-1, 2, 3, 4, 5 and 6 was determined in biopsies of skin from patients with psoriasis ($n=12$) and from normal skin ($n=12$). There were no differences in the intensity or localization of the adhesion molecules VLA-1, 2 and 4 and LFA-3. In contrast, VLA-3 and VLA-6, which are restricted to the basal keratinocytes in normal skin, were overexpressed in the spinous cells in psoriatic skin. ICAM-1 and VLA-5, which are not expressed by keratinocytes in normal skin, were focally induced, especially in cells above elongated rete ridges and where there was an infiltrate with intraepidermal granulocytes and lymphocytes.

In recent years there has been further understanding in the expression of cell-surface adhesion molecules that are involved in cellular interactions and with the extracellular matrix. The most important and best characterized are the integrin family,[1] several members of the immunoglobulin superfamily[2] and the so-called selectins.[3] There are other non-related molecules that have similar functions, such as membrane-bound proteoglycans, which can be added to this list (Table 1).[3] Whereas integrins are heterodimers with subfamily-specific β-chains that recognize defined peptide sequences on one or several other proteins or glycoproteins, the ligand structures of the selectins are less defined. There is evidence that adhesion molecules are critically involved in embryogenesis, tissue homeostasis, particularly in rapidly dividing tissues, as well as migration and homing events and in malignant proliferation of cells and metastases.

We have investigated as to whether there is aberrant expression of adhesion molecules in psoriasis, a chronic and relapsing skin disease with disturbances in epidermal proliferation and differentiation. We were interested as to whether members of the β_1-integrin family, that are primarily involved in cell–extracellular matrix interactions,[4] would show differences of expression on the keratinocytes in the involved psoriatic epidermis.

Methods

Skin biopsies

Twelve skin biopsies were obtained from patients with chronic plaque-type psoriasis from the centre of the plaque under local anaesthesia and prior to systemic or topical treatment. Informed consent was obtained from the patients. In addition, 12 biopsies were obtained from normal controls from plastic surgical excisions. The biopsies were all snap frozen in 0·9% NaCl with liquid nitrogen and then stored at −80°C. Serial 6-μm sections were cut from each block on a cryostat, air-dried for 24 h and then stored at −20°C until used.

Monoclonal antibodies

The ICAM-1 IgG1 RR1/1, the LFA-3 IgG1 TS2/9 and the VLA-α_1 IgG1 monoclonal antibodies were the gift from Dr T.A.Springer (Boston, U.S.A.). The VLA-β_1 IgG2b A-1A5 and the VLA-α_4 IgG1 B-5G10 monoclonal antibodies were the gift from Dr M.E.Hemler (Boston, U.S.A.). The VLA-α_3 J143 antibody was provided by Dr R.Kantor (New York, U.S.A.). The VLA-α_2 IgG1 10G11 and VLA-α_6 IgG2a GoH3 antibodies were the gift from Dr von dem Borne (Amsterdam) and Dr Sonnenberg (Amsterdam), respectively. The VLA-α_5 IgG1 BIE5 monoclonal antibody was the gift from Dr Damsky (San Francisco, U.S.A.). The horseradish peroxidase-conjugated rabbit anti-mouse Ig antibodies and goat anti-rabbit Ig antibodies were purchased from Dakopatts (Denmark) and Medac (Hamburg).

Indirect immunoperoxidase procedure

The immunohistochemical stainings were performed using a three-step immunoperoxidase technique.[5] The cryostat sections were fixed in acetone for 10 min at room temperature, air-dried and then incubated with

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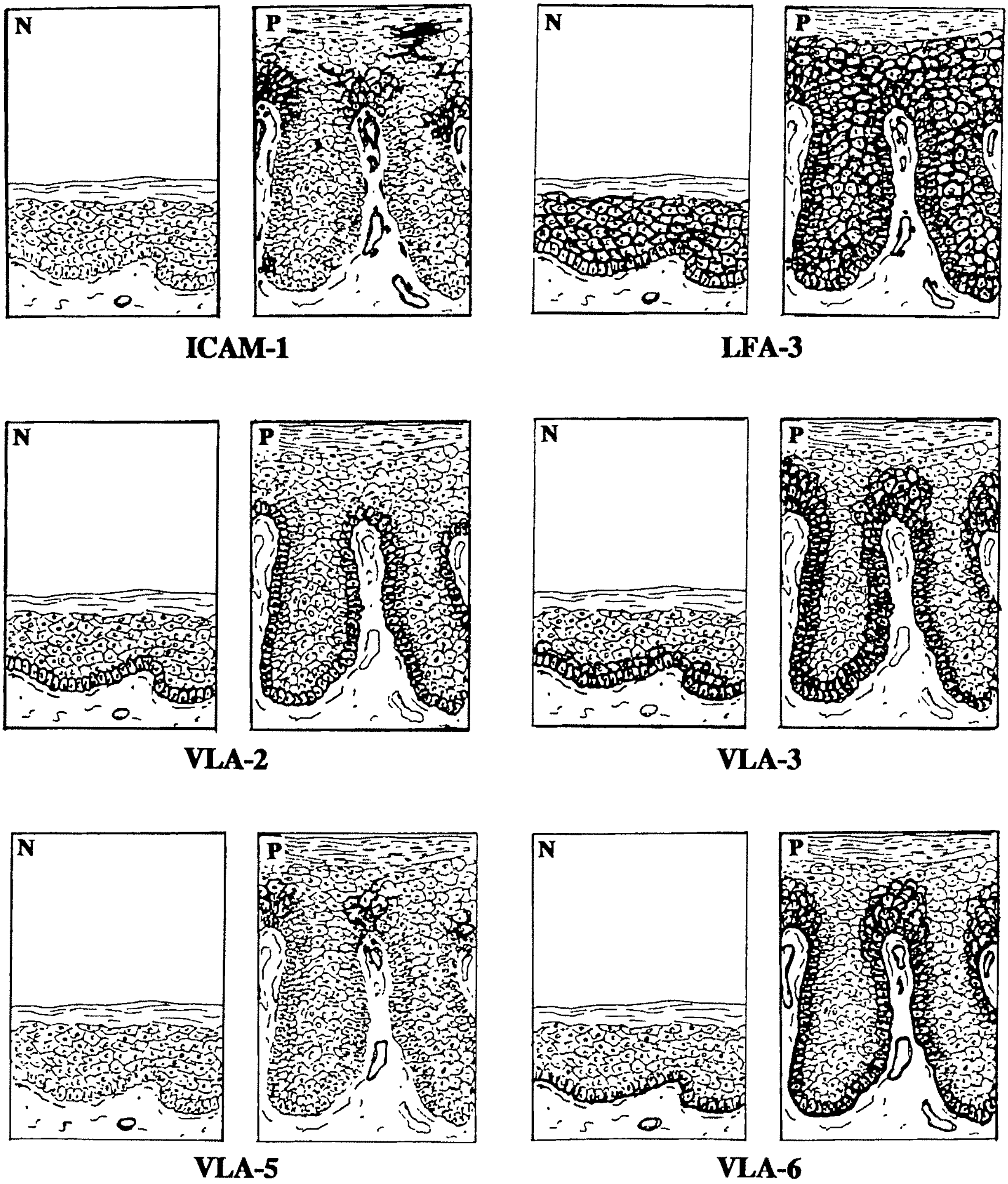

Figure 1. Schematic illustration of the distribution pattern of the investigated adhesion molecules in normal and psoriatic epidermis.

Table 1. Characteristics of adhesion molecules

Superfamily	Adhesion molecule	Molecular weight (kDa)		Known ligand
Integrins				
β_1-integrins	VLA-1	α_1:210	β_1:130	Coll I, IV, LM
	VLA-2	α_2:165		Coll I-IV, LM, FN
	VLA-3	α_3:135		LM, Coll I, FN
	VLA-4	α_4:150		FN, VCAM-1
	VLA-5	α_5:130		FN
	VLA-6	α_6:140		LM
β_2-integrins	LFA-1	α_L:180	β_2:95	ICAM-1, ICAM-2
	Mac-1	α_M:170		C3bi
	p150,95	α_X:150		C3bi
β_3-integrins	VNR	α_v:135	β_3:115	Vitronectin
	GPIIb-III	α_{IIb}:130		Vitronectin
Immunoglobulin superfamily	CD2	50		LFA3
	LFA-3	45–75		CD2
	ICAM-1	80–114		LFA-1
Selectins	ELAM-1	107–115		Sialylated Lewis x
	MEL-14	90		?
	CD62	140		CD15

the primary monoclonal antibody diluted to an optimal concentration in phosphate-buffered saline (PBS) at pH 7·4 and containing 1% bovine serum albumin for 30 min. After rinsing with NaCl/Tris buffer at pH 7·4, the sections were then incubated with peroxidase-conjugated rabbit anti-mouse IgG at a dilution of 1 : 15 with serum and PBS for 30 min. The slides were then rinsed twice in NaCl/Tris buffer and covered with peroxidase-conjugated goat anti-rabbit IgG at a dilution of 1 : 15 for 30 min. The sections were then incubated with 3,3′diaminobenzidine at a concentration of 0·6 mg/ml and 0·01% hydrogen peroxide at room temperature. The staining reaction was visually controlled and stopped by washing with the buffered saline. The sections were counterstained with haematoxylin for 10 min and then rinsed in tap-water. The sections were then mounted with coverslips using glycer-gel (Dakopatts, Hamburg). Appropriate controls were performed.

Results

The adhesion molecules VLA-1 and VLA-4 were not expressed either on normal or on the psoriatic keratinocytes. VLA-2 and LFA-3 showed the same pattern of expression in psoriasis as seen in normal skin (Fig. 1). In contrast, VLA-3, which is normally found in the basal layers of the epidermis, showed focal overexpression extending up to the stratum spinosum in the biopsies of psoriasis (Figs 1 and 2). A similar pattern could be

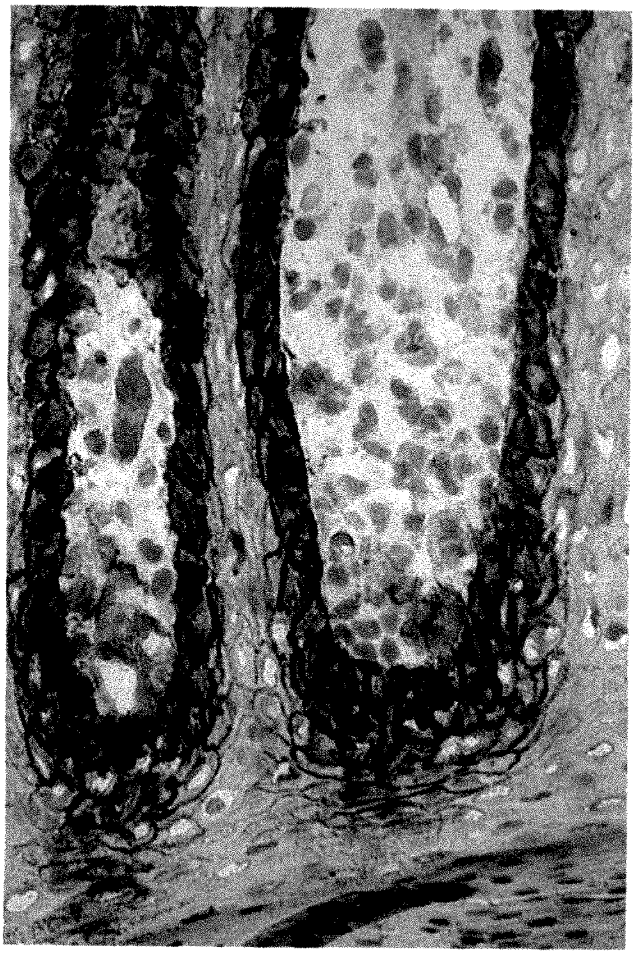

Figure 2. Focal over-expression of VLA-3 ($\alpha3$) on suprabasal psoriatic keratinocytes above rete ridges (× 400).

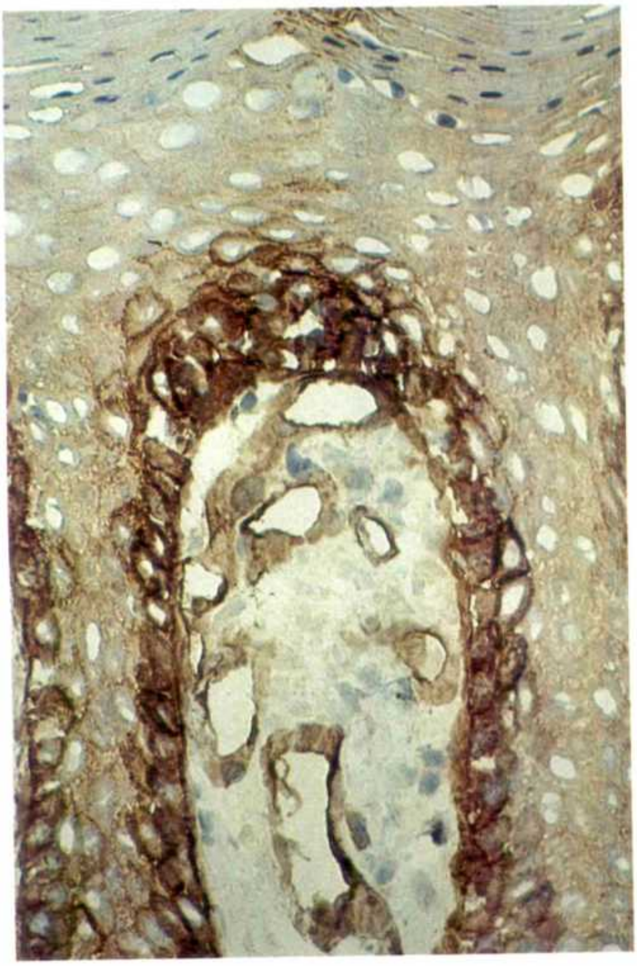

Figure 3. Expression of VLA-6 (α6) in psoriatic epidermis (× 400). The pattern is similar to the one of VLA-3 (α3).

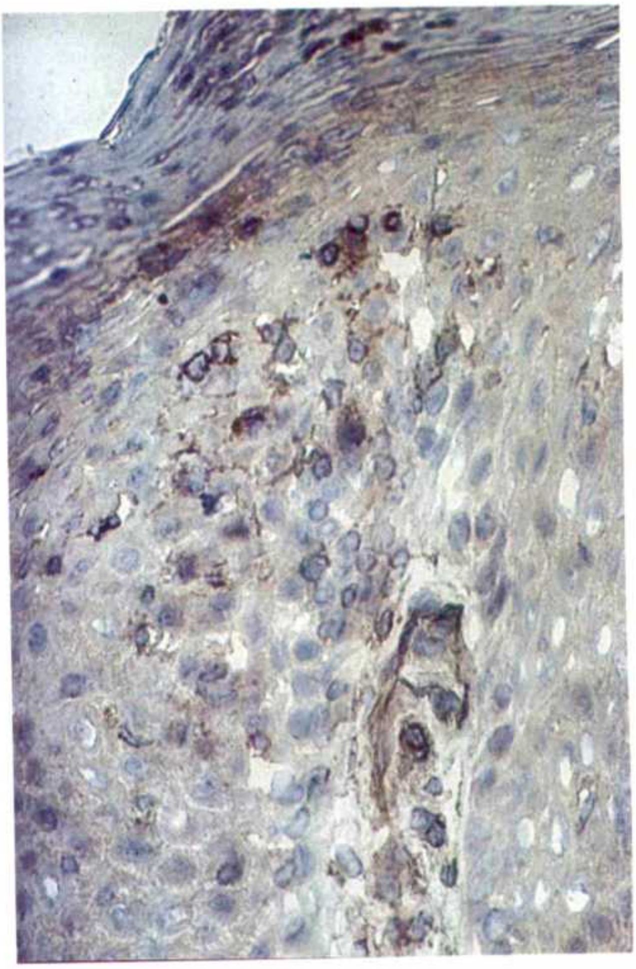

Figure 5. Focal expression of VLA-5 (α5) on psoriatic keratinocytes and infiltrating cells (× 400).

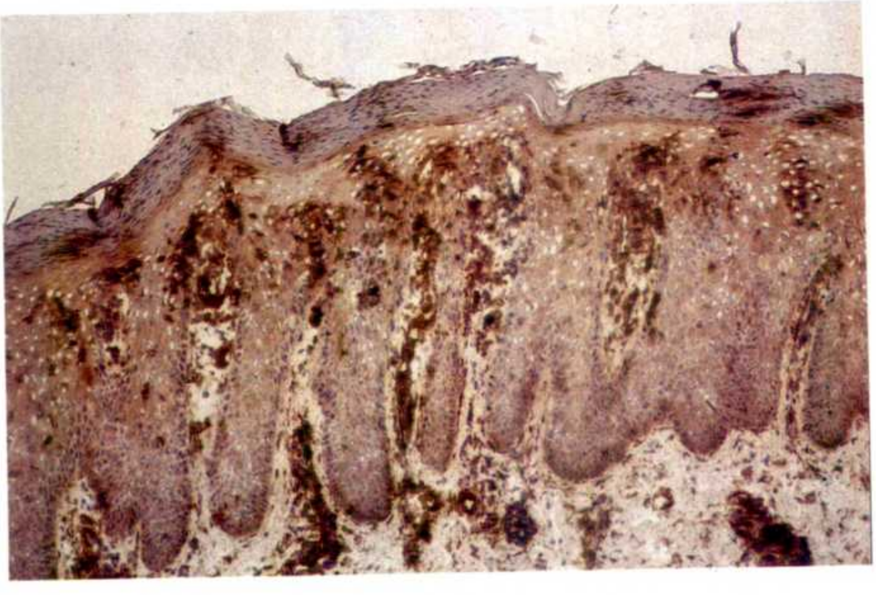

Figure 4. Focal ICAM-1-expression by psoriatic keratinocytes related to neighbouring intraepidermal leucocytes (× 100).

detected for VLA-6, which in normal skin is expressed in the basal cells with polarity towards the basement membrane. In the psoriatic skin there was focal overexpression of VLA-6 (Figs 1 and 3).

ICAM-1 and VLA-5 were completely absent in the normal epidermis, but there was focal induction of these molecules in the biopsies of psoriasis with pronounced staining for ICAM-1 (Figs 1 and 4) and weaker expression of VLA-5 (Figs 1 and 5). These molecules were also expressed on the dendritic cells in the epidermis. The overexpression of adhesion molecules VLA-3,-5 and -6 and ICAM-1 showed similar morphological patterns. They were mainly restricted to the epidermis overlying the elongated rete ridges and where intraepidermal lymphocytes and some granulocytes could be detected. No adhesion molecules were present around the intracorneal neutrophil microabcesses (Munroe's microabcesses) in the biopsies of psoriasis.

Discussion

Although a number of hypotheses have been suggested for the pathogenesis of psoriasis,[6,7] the exact mechanisms involved are still unclear. We have demonstrated that keratinocytes in psoriasis exhibit changes in the distribution of a number of adhesion molecules. Although VLA-1 is known to function as an extracellular matrix receptor for types I and IV collagen and laminin,[8] it is not expressed on epidermal keratinocytes in normal skin[9] or in various inflammatory cutaneous disorders.[10] We have found that VLA-1 is also absent on the keratinocytes in psoriatic epidermis. This indicates that VLA-1 differs in its cellular distribution from the ECM receptors VLA-2, VLA-3 and VLA-6, that also show laminin and collagen binding specificity and which are expressed on epidermal keratinocytes.

We found no expression of VLA-4 on the keratinocytes in both normal and diseased epidermis. VLA-4 is found predominantly on leucocytes and functions both as an extracellular matrix receptor for fibronectin, binding to the CS1 fragment and the Hep2 domain,[11,12] and as a cell-to-cell receptor to its ligand VCAM-1.[13] Functional experiments revealed that cultured keratinocytes do not recognize cell-binding sites within the CS1 region of the fibronectin molecule, suggesting that VLA-4 is not present on the surface of keratinocytes.[14]

LFA-3, the ligand of CD2,[15] was expressed on all keratinocytes of the basal and spinous layers in both normal and diseased skin. VLA-2, when expressed by keratinocytes, functions as a receptor for collagen, laminin and fibronectin.[16] As with LFA-3, VLA-2 retained its pattern of expression in the basal cell layer in psoriatic skin (Fig. 1).

In addition to their normal distribution, VLA-3 and VLA-6 were focally expressed in the keratinocytes of the spinous layer of psoriatic skin. This upregulation corresponded to the localization of intraepidermal mononuclear leucocytes and was most pronounced on the tips of the elongated psoriatic papillae. This was the region where intraepidermal fibronectin, the ligand of VLA-3 and VLA-5, could be demonstrated. While fibronectin is absent in the normal human epidermis, in psoriasis there is an enrichment of fibronectin focally in the stratum corneum between the basal and spinous keratinocytes above the rete ridges.[17] This intraepidermal fibronectin is produced by keratinocytes[18] and by Langerhans cells.

There was a weak focal expression of VLA-5 on the psoriatic keratinocytes and this upregulation was restricted to those keratinocytes adjacent to intraepidermal mononuclear leucocytes. VLA-5 is known to be the classical fibronectin receptor, recognizing the central cell-binding fibronectin domain with its RGD site.[19] Under physiological conditions VLA-5 is only expressed on epidermal keratinocytes during wound healing.[20] When re-epithelialization is complete, VLA-5 expression on the keratinocytes can no longer be detected.[21]

ICAM-1, the ligand of LFA-1,[22] which is expressed by more than 95% of infiltrating leucocytes in psoriasis, may play an important functional role in the trafficking of mononuclear leucocytes into the epidermis,[23] especially in inflammatory dermatoses.[24,25] However, it is not clear whether ICAM-1 is at first expressed on keratinocytes, and thus serves as a ligand for the invasion of mononuclear cells into the epidermis, or whether activated T-cells secrete γ-interferon and thereby induce ICAM-1 expression. As with VLA-5, the localization of intraepidermal leucocytes correlated well with the extent of the expression of ICAM-1 on the keratinocytes.

There are several possible explanations for the observed overexpression of β_1-integrins in psoriatic epidermis. The shortened epidermal transit time in psoriasis could delay the redistribution of molecules such as VLA-3, which *in vitro* are localized mainly at sites of cell attachment when anchored to the extracellular matrix, and afterwards are expressed on the entire cell surface.[16] This could result in the demonstration of VLA-3 in a higher part of the epidermis than in the normal skin. Also, there is increasing *in-vitro* evidence for the regulation of integrin expression by cytokines.[26] Cytokines with defined effects on β_1-integrin expression are transforming growth factor[27] and nerve growth factor,[28] while ICAM-1 expression is induced by γ-interferon, IL-1 and TNF.[29]

Finally, the failure of organized adhesion-molecule expression may be a basic defect in regulation and could be directly involved in the development and persistence of the lesions in psoriasis.

References

1 Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987; **48**: 549–54.

2 Dustin ML, Staunton DE, Springer TA. Supergene families meet in the immune system. *Immunol Today* 1988; **9**: 213–5.

3 Siegelman MH, Van de Rijn M, Weissman IL. Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science* 1989; **243**; 1165–72.

4 Hemler ME Adhesive protein receptors on hematopoetic cells. *Immunology Today* 1988; **9**: 109–13.

5 Sternberger LA. *Immunocytochemistry*. New York: J.Wiley, 1979.

6 Christophers E, Krueger G. Psoriasis. In: *Dermatology in General Medicine* (Fitzpatrick, ed.). 1987; 461–91.

7 Bos JD. The pathomechanisms of psoriasis; the skin immune system and cyclosporin. *Br J Dermatol* 1988; **118**: 141–55.

8 Kramer RH, Marks N. Identification of integrin collagen receptors on human melanoma cells. *J Biol Chem* 1989; **264**: 4684–8.

9 Konter U, Kellner I, Klein E *et al.* Adhesion molecule mapping in normal human skin. *Arch Dermatol Res* 1989; **281**: 454–62.

10 Kellner I, Konter U, Sterry W. Upregulation of adhesion molecules in inflammatory skin diseases. *Arch Dermatol Res* 1990; **281**: 550.

11 Hemler ME, Elices MJ, Parker C, Takada Y. Cell adhesion functions and structural aspects of VLA proteins in the integrin family. *J Cell Biochem* 1990; **14A**: 136.

12 Wayner EA. Characterization of the lymphocyte receptor for a new adhesion sequence, EILDVPST, located in the CS-1 domain of plasma fibronectin: function in mediating heterotypic lymphocyte adhesion. *J Cell Biochem* 1990; **14A**: 139.

13 Holzmann B, McIntyre BW, Weissmann IL. Identification of a murine Peyer's patch specific lymphocyte homing receptor as an integrin molecule with an alpha chain homologous to human VLA-4 alpha. *Cell* 1989; **56**: 37–46.

14 Adams JC, Watt FM. Changes in keratinocyte-extracellular matrix interactions during terminal differentiation: the role of fibronectin receptors. *J Cell Biochem* 1990; **14A**: 159.

15 Dustin ML, Sanders ME, Shaw S, Springer TA. Purified lymphocyte function-associated antigen 3 binds to CD2 and mediates T lymphocyte adhesion. *J Exp Med* 1987, **165**: 677–92.

16 Carter WG, Wayner EA, Bouchard S, Kaur P. Role of integrins $\alpha 2\beta 1$ and $\alpha 3\beta 1$ in cell–cell and cell–substrate adhesion of human epidermal cells. *J Cell Biochem* 1990; **14A**: 160.

17 Fyrand O. Studies on fibronectin in the skin II Indirect immunofluorescence studies in psoriasis vulgaris. *Arch Dermatol Res* 1979; **266**: 33–41.

18 Kubo M, Kan M, Isemura M *et al.* Effects of extracellular matrices on human keratinocyte adhesion and growth and on its secretion and deposition of fibronectin in culture. *J Invest Dermatol* 1987; **88**: 594–601.

19 Yamada KM, Aota S-I, Akiyama SK. Fibronectin and cell interactions. *J Cell Biochem* 1990; **14A**: 135.

20 Toda KI, Grinnell F. Activation of human keratinocyte fibronectin receptor function in relation to other ligand-receptor interactions. *J Invest Dermatol* 1987; **88**: 412–7.

21 Clark RAF. Integrin mapping of cutaneous wounds. *J Cell Biochem* 1990; **14A**: 147.

22 Staunton DE, Marlin SD, Stratowa C *et al.* Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell* 1988; **52**: 925–33.

23 Nickoloff BJ. Role of interferon-γ in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. *Arch Dermatol* 1988; **124**: 1835–42.

24 Lange Wantzin G, Ralfkiaer E, Lisby S, Rothlein R. The role of intercellular adhesion molecules in inflammatory skin reactions. *Br J Dermatol* 1988; **119**, 141–5.

25 Lisby S, Ralfkiaer E, Rothlein R, Vejlsgaard GL Intercellular adhesion molecule-I (ICAM-I) expression correlated to inflammation. *Br J Dermatol* 1989; **120**: 479–84.

26 Springer TA. Adhesion receptors of the immune system. *Nature* 1990; **346**: 425–34.

27 Heino J, Ignotz RA, Hemler ME *et al.* Regulation of cell adhesion receptors by transforming growth factor-β. *J Biol Chem* 1988; **264**: 380–8.

28 Dedhar S. Differential regulation of expression of specific integrin receptors by TGF-β and NGF on neuroblastoma cells. *J Cell Biochem* 1990; **14A**: 162.

29 Dustin MI, Rothlein R, Bhan AK *et al.* Induction by IL-1 and interferon γ: tissue distribution, biochemistry and function of a natural adherence molecule (ICAM-1). *J Immunol* 1986; **137**: 245–54.

British Journal of Dermatology (1991) **125**, 217–221. ADONIS 000709639100204Q

The metabolism of fibroblasts from normal and fibrotic skin is inhibited by minoxidil *in vitro*

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Accepted for publication 27 March 1991

Summary

The effects of minoxidil *in vitro* were studied using fibroblasts grown from the lesional skin of patients with lichen sclerosus et atrophicus, morphoea and from the skin of normal individuals. The proliferation of all fibroblast lines over 3 days was inhibited in proportion to the concentration of minoxidil, being 20% or less of controls at 1 mM, where cell viability was only marginally reduced (84 ± 2% vs. 88 ± 2% (SEM) in controls). At 5 mM there was usually a net loss of cells and only 72% of those remaining were viable. In contrast, minoxidil at 0·1–1 mM stimulated the proliferation of foreskin keratinocytes by up to 130%. Contraction of collagen lattices containing the three types of fibroblasts was inhibited by 22–26% with 1 mM minoxidil after 5 days and by 50–94% with 5 mM. Secretion of glycosaminoglycans by normal fibroblasts showed concentration-dependent reduction, being 25 ± 6% of that of untreated cultures with 1 mM minoxidil. These findings show that minoxidil has a range of inhibitory effects on both normal and abnormal skin fibroblasts *in vitro*, which contrast with its stimulation of skin epithelial cells, and support suggestions that it may provide a useful topical treatment for keloids and other fibroses.

The hypertrichosis associated with the systemic use of minoxidil to control hypertension[1] suggested that this drug would be of help in the treatment of baldness.[2] Cell-culture studies have demonstrated that minoxidil has direct effects on epidermal cells *in vitro*[3,4] independent of the blood supply. More recently Murad and Pinnell[5] found a selective inhibition of the enzyme lysyl hydroxylase in cultured skin fibroblasts and that prolyl hydroxylase was unaffected. As hydroxylation of lysine residues is the first step in collagen cross-linking, the authors suggested that minoxidil might be an effective topical treatment for fibroses, such as keloids and hypertrophic scars, where collagen accumulates. We have now extended some of the observations on normal skin fibroblasts to cells from fibrotic skin.

Methods

Materials

Minoxidil was provided by Upjohn Limited, Crawley, U.K. The white crystalline powder was dissolved in culture medium to give a 5 mM solution, filtered to ensure sterility and diluted with fresh culture medium to provide a range of concentrations. Culture media and supplements were obtained from Gibco, Paisley, Scotland, unless specified otherwise.

Fibroblast culture

Fibroblast lines were established from excision biopsies of the lesional skin of patients with morphoea, extragenital lichen sclerosus et atrophicus (LSA), and from the normal forearm skin of healthy volunteers. The biopsies were washed, chopped into 1-mm fragments and sandwiched between a glass coverslip and the bottom of a six-well plastic dish (Nunc) in Dulbecco's Eagle medium supplemented with 20% fetal calf serum (FCS), penicillin, streptomycin and glutamine. The dishes were cultured at 37°C in an atmosphere of 5% CO_2: 95% air. The medium was changed twice weekly and after 6–8 weeks, when there was sufficient growth of fibroblasts, the cells were dispersed with typsin-versene and transferred to 25-cm^2 flasks in the same medium with 10% FCS. Samples of third and later passage cells were preserved in liquid nitrogen until needed, and then recovered for use in experiments as 5th to 10th passage cells.

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Presented at the meeting of the British Society for Investigative Dermatology in Newcastle, September, 1990.

Fibroblast proliferation assay

Twenty-eight cultures were set up by plating 10^5 fibroblasts in each 25-cm^2 flask (day 0) in medium containing 10% FCS. The medium was changed on day 1 and on day 3 four flasks were used for baseline cell counts. The cultures were washed with phosphate-buffered saline (PBS) and cell suspensions prepared with trypsin-versene were counted electronically (Coulter Counter DN). The remaining cultures were grouped in fours to receive medium containing 0·1, 0·2, 0·5, 1 and 5 mM minoxidil, leaving a second control group of four cultures untreated. In two experiments shown in Figure 1, the total number of cultures was increased to 32 to accommodate an additional minoxidil concentration. Media were replaced on day 4 and on day 6 the cells in all cultures were counted. The increase in total cells in each group was expressed as a percentage of that in the untreated controls.

Collagen lattice contraction

Collagen lattices were made with type 1 collagen from rat-tail tendons, as previously described,[6] in the 35-mm diameter wells of bacteriological grade six-well dishes (Linbro, Flow Laboratories). Each lattice contained 2×10^5 fibroblasts and measurements of lattice diameter were made daily using mm-squared graph paper over a light box. Two lattices were used for each concentration of minoxidil, which was included in the lattice mix at zero time.

Glycosaminoglycans secretion

Culture media collected on day 6 of fibroblast proliferation assays were dialysed against acetate buffer (pH 5), concentrated twofold with polyacrylamide beads, digested overnight at 37°C with testicular hyaluronidase (Sigma Type 1; 2 mg per sample) and acidified with trichloracetic acid to a final 5% to precipitate protein. The uronic acid in 40,000 × gmax supernatants was detected colorimetrically with *m*-hydroxydiphenyl at 520 nm after boiling with sulphuric acid-borate.[7] Glycosaminoglycans (GAG) secretion was expressed as μg uronic acid secreted per 10^7 fibroblasts in 48 h and calculated as a percentage of that in untreated control cultures.[8]

Keratinocyte proliferation assay

Foreskins obtained from normal infants were treated with dispase (Sigma, protease type IX) at 4°C overnight to separate the epidermis from the dermis. The epidermis was incubated in 0·25% trypsin in PBS at 37°C for 30 min to release the epidermal cells. The cells were collected by centrifugation, counted and plated in 24-well plastic dishes (Nunc) in Dulbecco's Eagle medium diluted 3:1 with Ham's F12 medium and containing 10% FCS, penicillin, streptomycin, 10^{-10} M cholera toxin (Sigma), 5 μg/ml transferrin and 2×10^{-9} M tri-iodothyronine. Plating densities of the crude epidermal suspension were approximately 7×10^5 cells per well. After 2 days at 37°C in 5% CO_2: 95% air, the culture medium was supplemented with 10 ng/ml epidermal growth factor (EGF). After a further 2 days, four wells of the dish were trypsinized for baseline cell counts while other wells were replenished with standard medium (four wells) as controls, or with 0·1–5 mM minoxidil (four wells per concentration) dissolved in standard medium without EGF. Media were replaced the next day and after a final 48 h all the cultures were trypsinized for cell counts. Proliferation in minoxidil-treated wells (i.e. increase in cells above baseline counts) was expressed as a percentage of that in untreated control wells.

Results

Cell proliferation

The proliferation of five lines of normal fibroblasts, one line from morphoea and one from LSA was similarly affected by minoxidil. In general, there was concentration-dependent inhibition of proliferation beginning at 0·1 mM (Figs 1 and 2), as described by Murad and Pinnell.[5] Proliferation was less than 25% of untreated

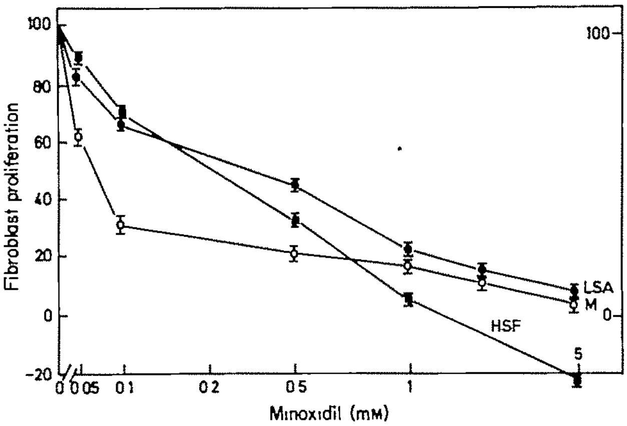

Figure 1. Effect of minoxidil on the proliferation of fibroblasts from lesions of morphoea, LSA and from normal skin (HSF). Each point is a mean from four cultures ± SE.

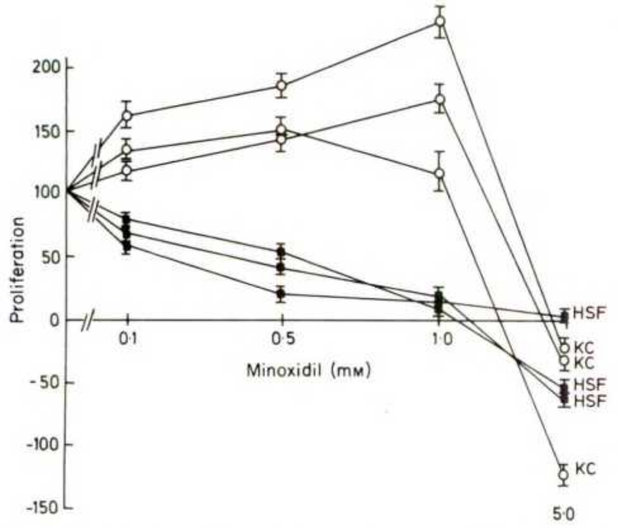

Figure 2. Comparison of the inhibitory effect of minoxidil on the proliferation of three additional lines of fibroblasts from normal skin (HSF, ■ means ± standard errors, $n=4$), and its stimulation of three different preparations of foreskin keratinocytes (KC, ○, means ± SE, $n=4$).

controls at 1 mM and approached zero or showed a net loss of cells at 5 mM. In a minority of experiments (4/10) there was some (<50%) stimulation of proliferation with 0·1 mM minoxidil, but this effect was not seen when the experiments were repeated. Different responses from the three cell lines in Figure 1, for example the greater sensitivity of the morphoea fibroblasts at 0·1 mM minoxidil, are not necessarily representative of all cell lines of that origin. Figure 2 displays similarly wide variation in three lines from normal skin.

Three experiments with keratinocytes from different foreskins gave similar results (Fig. 2). At minoxidil concentrations of 0·1, 0·5 and 1 mM, proliferation was increased by averages of 39, 60 and 77%, respectively, but there was a net loss of cells at 5 mM, indicating cytotoxicity.

Viabilities of the fibroblasts, judged by trypan blue exclusion, remained essentially unchanged except at 5 mM, where some 80% of the cells removed in the pre-trypsin rinse were dead and only 71% of those recovered with trypsin-versene for counting were viable, compared to 88% in untreated controls ($P<0{\cdot}05$; *t*-test with 4 d.f.). Phase-contrast microscopy on day 6, after 3 days of exposure to minoxidil, showed cells of normal appearance but fewer in number at 1 mM, whereas many of the cells looked abnormal or dead in 5 mM minoxidil (Fig. 3). Such cultures were, however, able to resume proliferation if returned to medium without minoxidil for a further 3 days (results not shown).

Collagen lattice contraction

Lattices were set up with one line of fibroblasts from each group of cell lines and were observed for 5 days. Results

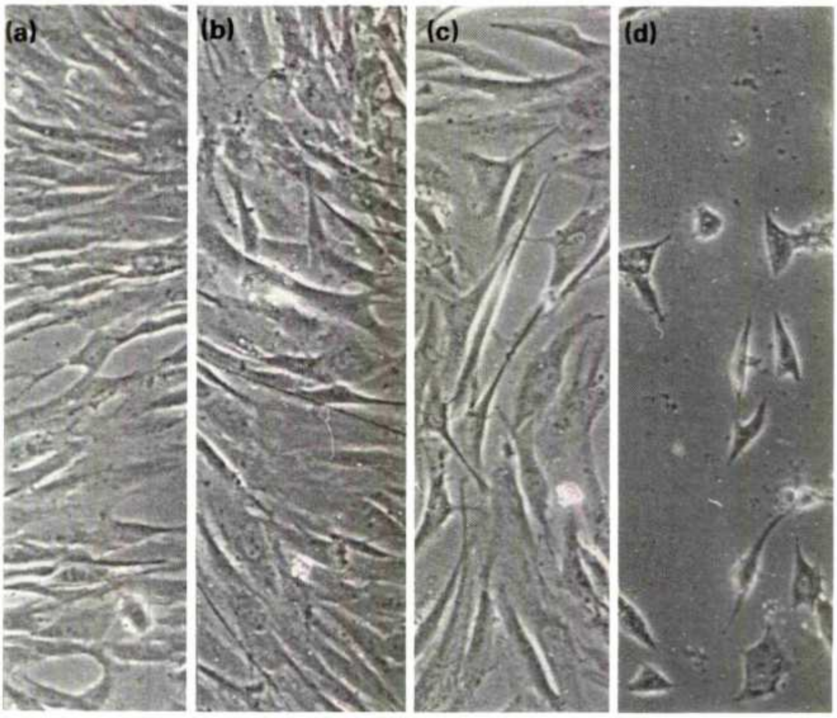

Figure 3. Phase contrast photomicrographs of normal skin fibroblasts after 3 days' incubation with different concentrations of minoxidil. With 1 mM cells are sparse but healthy, while many of those remaining in 5 mM are dead or damaged. (a) controls; (b) 0·5 mM; (c) 1 mM; (d) 5 mM (×125).

Table 1. Inhibition of collagen lattice contraction by minoxidil

Minoxidil (mM)	Percentage inhibition after 5 days			
	HSF	M	LSA	Mean
0·05	5	15	5	8 ± 3
0·1	16	20	11	16 ± 3
0·5	16	20	17	18 ± 1
1·0	26	25	22	24 ± 1
5·0	79	50	94	74 ± 13

The three fibroblast lines were from normal skin (HSF), morphoea (M) and lichen sclerosus et atrophicus (LSA). Each value is a mean from two lattices and overall mean values at each drug concentration are shown with standard errors.

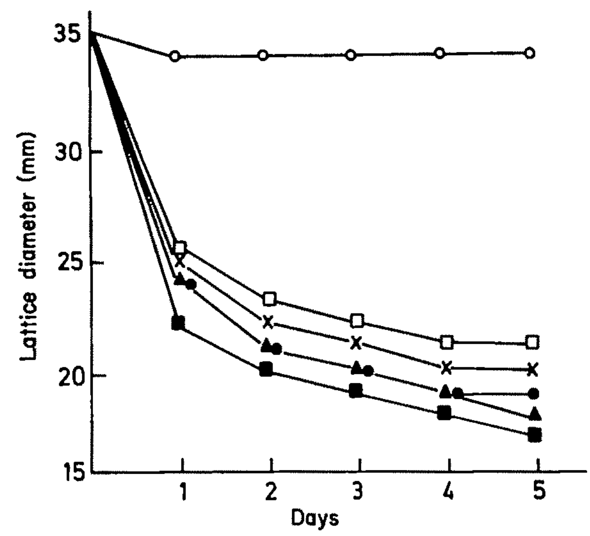

Figure 4. Contraction of collagen lattices by LSA skin fibroblasts in the presence of minoxidil. Each value is a mean from two lattices. ○, 5 mM; □, 1 mM; x, 0·5 mM; ●, 0·1 mM; ▲, 0·05 mM; ■, controls.

from the three lines were similar, except at 5 mM minoxidil, where the morphoea fibroblasts were less affected than the other two lines (Table 1). In general, contraction was inhibited in proportion to the concentration of minoxidil (Fig. 4). The effect was apparent at 0·05 mM and at 1 mM contraction was inhibited by 22–26% by day 5 compared to that in untreated lattices. A similar finding has recently been reported from another laboratory.[9]

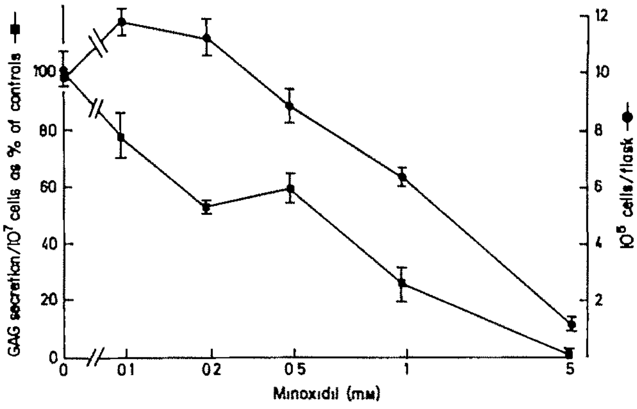

Figure 5. Inhibition of GAG secretion in normal skin fibroblasts by minoxidil. Each point is a mean from four cultures$\pm$SE.

GAG secretion

Assessment of the effect of minoxidil on one line of normal skin fibroblasts showed an inhibition of the secretion of GAG in proportion to minoxidil concentration (Fig. 5). Control cultures ($9·8\times10^5$ cells per flask) secreted 142 μg GAG-uronic acid per 10^7 cells. This was reduced by 24% at 0·1 mM and by 74% at 1 mM minoxidil, compared to that in untreated cultures, reaching zero at 5 mM ($r=-0·906$ for log drug concentration versus percentage GAG recovery; $P<0·001$ with 18 d.f.). In a line of morphoea fibroblasts secreting 98 μg GAG-uronic acid per 10^7 cells at a mean density of $6·9\times10^5$ cells per flask, secretion was reduced to $23\pm15\%$, $18\pm9\%$ and $42\pm18\%$ in 0·2, 0·5 and 1 mM minoxidil, respectively.

Discussion

An ideal antifibrotic compound would act at a specific point in the synthesis of procollagen or in the assembly of collagen fibrils and would not be toxic to fibroblasts or epidermal cells.[10] It would be an advantage if the drug also inhibited GAG secretion, because these macromolecules may contribute to thickening and hardening of the skin through their interaction with collagen or their water-binding capacity. Many compounds with promising activities against collagen synthesis are disqualified from use by their toxicity[10] but minoxidil appears to fulfil all the criteria. It modulates a range of fibroblast activities without toxic effects on the cells detectable by phase-contrast microscopy or trypan-blue exclusion. The considerable experience of minoxidil available from its uses for hypertension and for alopecia is a further advantage.[1,2,11] The possible side-effect of increased hairiness on the treated skin needs to be considered, but is probably acceptable, particularly because it would not persist when treatment ended.

Although we have observed inhibition of fibroblast GAG secretion *in vitro* with a range of other drugs, including griseofulvin, salicylate, D-penicillamine and *p*-aminobenzoate,[12–14] the degree of inhibition with minoxidil is more impressive. The 75% inhibition at 1 mM (Fig. 5), for example, compares with the effect of 10 μg/ml clobetasol propionate,[15] a potent corticosteroid. Interpretation of Figure 5 is complicated by the inverse relationship between GAG secretion per cell and cell density (cells/flask).[8] However, because the lower number of cells present at 1 mM minoxidil, compared to controls, would lead to greater GAG secretion per cell if there was no direct effect of minoxidil on GAG secretion, the large degree of inhibition actually observed is even more remarkable. The large scale of the inhibition suggests that secretion of both hyaluronate and sulphated GAG is affected by minoxidil. The secreted GAG is mainly hyaluronic acid;[8] an estimate of 86% with only 14% sulphated GAG was obtained from this normal fibroblast line in another study.[16] Our colorimetric reagent measures all forms of uronic acid.[7] Minoxidil must therefore affect hyaluronic acid secretion, and the complete blocking of secretion in the normal fibroblasts with 5 mM minoxidil suggests that the sulphated GAG was also affected, although this argument is weakened by the cytotoxicity seen at this concentration. The qualitative nature of the effect of minoxidil on GAG requires further study.

It is tempting to regard the selective stimulation of keratinocyte proliferation by minoxidil as being related to its effect on hair follicle epithelial cells, although the precise mechanism is uncertain. Current evidence suggests that minoxidil prolongs the time that follicles spend in the anagen phase of the hair growth cycle, just as it seems to extend the growth phase of cultured keratinocytes.[3,4]

Our experiments define the range of concentrations at which minoxidil is active *in vitro*, namely 0·05–5 mM. Minoxidil was cytotoxic at 5 mM, but this probably far exceeds the concentration that can be achieved *in vivo*. Data on tissue concentrations are not available, but blood levels are very low, being <10 ng/ml with topical application and up to 60 ng/ml in patients taking 5 mg/day orally.[11] These concentrations compare with 5–10 μg/ml acting on mouse keratinocytes *in vitro*,[3] 70 μg/ml affecting human keratinocytes *in vitro*[2] and 0·05–0·5 mM (approximately 10–100 μg/ml) inhibiting lysyl hydroxylase *in vitro*,[4] which resembles the range employed in the present work. These figures present the only problem in the topical use of minoxidil in the treatment of fibrotic conditions. It has the right range of activities and is not cytotoxic, but effective concentrations may not be achieved *in vivo*. Clinical trials are required to determine this, especially as active concentration ranges *in vivo* and *in vitro* may not be identical.

References

1 Daigie HJ, Dollery CT, Daniel J. Minoxidil in resistant hypertension. *Lancet* 1977; **2:** 515–8.

2 Olsen EA, Weiner MS, Delong ER, Pinnell SR. Topical minoxidil in early male pattern baldness. *J Am Acad Dermatol* 1985; **13:** 185–92.

3 Baden HP, Kubilus J. Effect of minoxidil on cultured keratinocytes. *J Invest Dermatol* 1983; **81:** 558–60.

4 Cohen RL, Alves MEAF, Weiss VC *et al.* Direct effects of minoxidil on epidermal cells in culture. *J Invest Dermatol* 1984; **82:** 90–3.

5 Murad S, Pinnell SR. Suppression of fibroblast proliferation and lysyl hydroxylase activity by minoxidil. *J Biol Chem* 1987; **262:** 11973–8.

6 Adams LW, Priestley GC. Contraction of collagen lattices by skin fibroblasts: drug-induced changes. *Arch Dermatol Res* 1988; **280:** 114–8.

7 Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronic acids. *Anal Biochem* 1973; **54:** 484–9.

8 Oakley CA, Priestley GC. Density-dependent regulation of skin fibroblast glycosaminoglycans *in vitro*: control by a secreted factor. *Arch Dermatol Res* 1985; **227:** 264–9.

9 Cherry G, Hughes MA, Dawber RPR, Ryan TJ. Minoxidil-induced changes in the contraction of collagen lattices by human skin fibroblasts: a new means of control of excessive clinical scar formation. *J Invest Dermatol* 1990; **94:** 514.

10 Levene CI. Possibilities for the therapeutic control of fibrosis. *Br J Dermatol* 1985; **112:** 363–71.

11 Spindler JR. Deaths occurring during clinical studies of topical minoxidil. *J Am Acad Dermatol* 1987; **16:** 725–9.

12 Priestley GC, Brown JC. Effects of griseofulvin on the morphology, growth and metabolism of fibroblasts in culture. *Br J Dermatol* 1978; **99:** 245–52.

13 Priestley GC. Changes in the growth and metabolism of cells cultured from normal, sclerotic and rheumatoid connective tissue brought about by D-penicillamine and by sodium salicylate. *J Invest Dermatol* 1980; **74:** 413–7.

14 Priestley GC, Brown JC. Effects of potassium para-aminobenzoate on growth and macromolecule synthesis on fibroblasts cultures from normal and sclerodermatous human skin and rheumatoid synovial cells. *J Invest Dermatol* 1979; **72:** 161–4.

15 Priestley GC. Effects of corticosteroids on the growth and metabolism of fibroblasts cultured from human skin. *Br J Dermatol* 1978; **99:** 253–61.

16 Priestley GC. Glycosaminoglycans production by cultured skin fibroblasts from the Pasini and Cockayne–Touraine forms of dominant dystrophic epidermolysis bullosa. *J Invest Dermatol* 1991; **96:** 168–71.

British Journal of Dermatology (1991) 125, 222–226. ADONIS 0007096391002055

Ultraviolet light induction of peripheral granulocytosis with splenomegaly: protection of mice with topical *p*-aminobenzoic acid (PABA)

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Accepted for publication 27 February 1991

Summary Hairless mice were exposed to UVB irradiation from a Philips T1 12 light source. Mice that were exposed to UV-light and not protected with 5% *p*-aminobenzoic acid (PABA) showed a significantly higher number of peripheral blood granulocytes ($P<0\cdot001$) and a significantly higher mean weight of both the spleen and the liver ($P<0\cdot001$) than the non-irradiated controls. Light microscopy of the histology of the enlarged liver and spleen showed a proliferation of granulocytes and the reticuloendothelial cells. Treatment with topical PABA during the whole period of UV-exposure prevented the peripheral blood granulocytosis. These protected mice also had a significantly ($P<0\cdot001$) lower mean weight for the liver and spleen than UV-exposed and non-protected mice.

Malignant transformation of epidermal cells by ultraviolet B (UVB) irradiation is well documented[1,2] and, in experimental mice, is preceded by the gradual development of epithelial hyperplasia.[2] UVB also affects bone-marrow derived cells in the skin such as Langerhans cells, lymphocytes and monocytes.[3–13] In mice, suppressor mechanisms are induced which inhibit the rejection of transplanted syngeneic UVB-induced tumours.[3] The expression of surface markers on the Langerhans cells is altered and reduced[6–8] with loss of contact hypersensitivity.[8] External UVB irradiation may even induce lymphatic leukaemia in mice.[9,10] In man, UVB irradiation has been observed to inhibit lymphocyte blastogenesis and monocyte function,[4,5] reducing the number of circulating T cells[9] and altering the T-cell subset ratios.[12]

About 10–30% of UVB and 40–50% of UVA is transmitted to the dermis in Caucasian skin[14] and can produce degenerative changes and solar elastosis with chronic exposure. In hairless mice UVB can induce similar changes.[15,16] We have studied the effect of UVB on circulating white blood cells as part of an investigation of its photocarcinogenic effects on the skin of hairless mice.[17] We have also investigated the photoprotective effect of topically applied PABA on these animals.

Methods

Female hairless and lightly pigmented (Hr/Hr) mice (Bomholdsgaard, Denmark) were 8–12 weeks old when used at the beginning of the study. The mice were randomly divided into four groups of 30 and the treatments used are shown in Table 1. A solution of a 5% *p*-aminobenzoic acid (PABA) in a vehicle consisting of 70% ethanol and 5% glycerol in water was used in this study and its absorption spectrum is shown in Figure 1.

The UV source was a Corona-2 lamp (Sweden), equipped with a bank of three Philips T1 40 W/12 light tubes and the irradiance, measured with a Centra UV-meter (Osram®), was 0·86 mW/cm^2 UVB (280–320 nm) and 0·1 mW/cm^2 UVA (320–400 nm) at a distance of 70 cm. UVC (250–280 nm) was measured using a UVX radiometer (UVP Inc, CA, U.S.A.) and was found to be 0·06 mW/cm^2 at a distance of 70 cm. The emission spectrum of the sunlamp is shown in Figure 2.

The minimum erythema dose (MED) for this strain of mouse was 175 mJ/cm^2. The suberythemal starting dose was 155 mJ/cm^2 and was increased by 25–30% every second week during the first 8 weeks and then remained constant at 360 mJ/cm^2 UVB, corresponding to an increase in radiation time of 3–7 min. The total UVB dose was approximately 49 J/cm^2. Immediately before each UV-exposure the PABA sunscreen was applied to the

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Table 1. Treatment schedules for four groups of 30 mice

Group	Local treatment	UVR
A	None	None
B	None	Week 1–30
C*	PABA week 16–26	Week 1–30
C	PABA week 1–30	Week 1–30

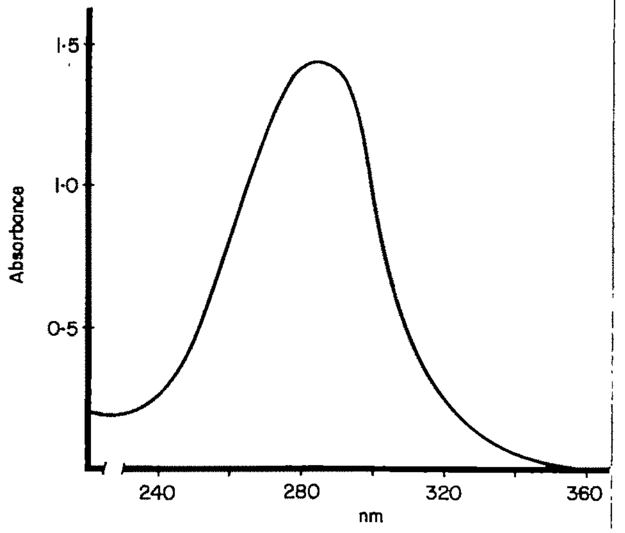

Figure 1. The absorption spectrum of 5 × 10^{-5} M *p*-aminobenzoic acid.

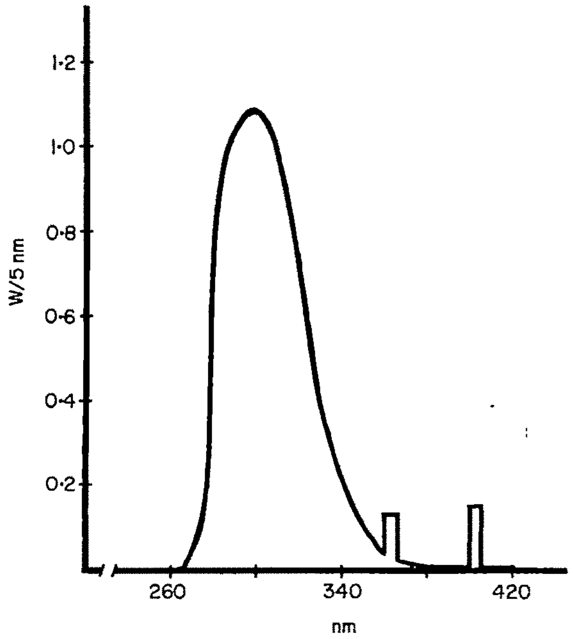

Figure 2. The emission spectrum of Philips T1 12 light tubes.

skin on the backs of the mice in Groups C* and C. The animals were irradiated 5 days per week over 30 weeks. Due to a 30% lower UV-intensity at the ends of the cages as compared to the middle, the cages were rotated before each daily treatment. The post-irradiation observation time was 10 weeks.

When aged 48–52 weeks, each mouse was anaesthetized by intraperitoneal injection of midazolam (Dormicum®) and about 1 ml of blood collected from the femoral vein. Blood smears were Giemsa-stained and the blood samples were immediately analysed in a Cell-Dyn 400 (Sequonia-Turner®) and the serum in a Cobra Mira (Roche®). The samples from all groups were analysed in parallel. The animals were then killed and the liver and spleen removed and weighed. Specimens of bone marrow were obtained from the left femur. Sections of skin tumour, liver, spleen and bone marrow from the mice were stained with haematoxylin and eosin and studied using light microscopy.

Statistics

The results were expressed as means ± SEM. The groups were compared using a Kruskal–Wallis test with a 5% significance level.[18] Two groups were compared with each other using a Welsh test and the Bonferronis correction for repeated testing.[19] Differences were considered statistically significant when *P*-values were $\leqslant 0{\cdot}05$.

Results

Skin

Two mice in Group B and one in Group C died during the study. In the UV-exposed groups the number of skin tumours ranged from one to eight per animal. In the UV-exposed Group B, 25 out of 28 animals had squamous cell carcinomas (SCC), whereas 11 out of 30 partially protected mice (Group C*) had SCC. In those mice that were protected (Group C) one had a SCC and no tumours developed in the controls (Group A).[17] UV-exposure induced tanning that was reduced considerably by PABA.

Blood analyses

The UV-exposed and non-protected animals (Group B) had a significantly lower concentration of haemoglobin than those in the other groups ($P < 0{\cdot}001$). UV-exposed, part-time-protected animals (Group C*) and UV-exposed

Table 2. Blood analyses from four groups of 30 mice

Groups	A	B	C*	C
Hb (g/100 ml)	13·6±0·18	11·7±0·55	13·6±0·32	14·4±0·15
WBC (10^9/l)	7·1±0·4	20·1±4·6	13·2±2·8	6·3±0·5
ALAT (μmol/l)	30±1·9	37±7·0	28±3·3	27±1·9
Creatinine (μmol/l)	135±7	82±3	92±8	118±6

Table 3. The weight of spleens and livers from four groups of 30 mice

Groups	A	B	C*	C
Spleen (g)	0·17±0·01	0·53±0·04	0·27±0·03	0·15±0·01
Liver (g)	1·81±0·05	1·98±0·06	1·77±0·05	1·64±0·03

A, Controls; B, UV-exposed mice; C*, UV-exposed part-time-protected mice; C, UV-exposed full-time-protected mice.

full-time-protected animals (Group C) did not differ significantly in this respect from the non-irradiated controls (Table 2).

UV-exposed non-protected mice (Group B) and part-time-protected mice (Group C*) showed a significantly higher number of peripheral white blood cells (WBC) than non-irradiated controls (Group A) ($P<0{\cdot}001$). The proportion of lymphocytes and mononuclear cells did not differ significantly between the groups.

While the serum concentration of creatinine was significantly lower in the UV-exposed non-protected mice and UV-exposed part-time-protected mice ($P<0{\cdot}001$), the concentration of serum alanine aminotransferase did not differ significantly between the groups.

Mean weight of spleen and liver

The spleens of the UV-exposed non-protected mice (Group B) and part-time-protected mice (Group C*) were significantly heavier than those of the controls (Group A) and UV-exposed full-time-protected mice (Group C). The spleens of the latter had a significantly lower mean weight than those of the controls (Group A) ($P<0{\cdot}001$; Table 3).

The livers of UV-exposed non-protected mice (Group B) had a significantly higher mean weight compared with the other groups ($P<0{\cdot}001$) among which there was no significant difference.

Histology

On light microscopy there was extramedullary haemopoiesis in the spleens even in non-irradiated mice, but this was more marked in the irradiated animals (Group B), with granulocytosis throughout the red pulp and predominantly in the central areas. The liver sinusoids of some irradiated mice (Group B) were also infiltrated with granulocytes and the bone marrow had a moderately enhanced content of granulocytes.

One UV-exposed mouse (Group B) had grossly enlarged lymph nodes, spleen and liver and 100×10^9/l of immature leucocytes in the blood. The bone marrow of this animal showed a moderate infiltration with immature lymphoid cells and the architecture of the spleen was disturbed by these infiltrating cells. The liver and kidneys also had a moderate perivascular infiltrate with these immature lymphoid cells.

The UV-exposed full-time-protected mice (Group C) and part-time-protected mice (Group C*) showed no significant differences from the controls.

Discussion

The mice used in this study were T-cell competent and lightly pigmented, and compared with nude albino strains resembled more human Caucasian skin. The emission spectrum of our sunlamp however differed somewhat from that of sunlight. Stockholm is at a latitude of 59°N and Wester[20] found that in sunlight there was about 48 times as much UVA than UVB. Our lamp emitted more UVB than UVA as well as small amounts of UVC. In Caucasian skin the amount of UV rays penetrating to the corium varies from as little as 1% at 250 nm up to 44% at 320 nm.[14] The skin in mice is much thinner than in humans and UVC may have also influenced this study.

Laboratory mice show a strong granulocyte response to a number of conditions,[21,22] including irradiation with UVB.[9] Humans however show a very transient granulocytosis after UVB irradiation.[23] The mouse used in this study provides a sensitive model for the granulocytic response to UV irradiation. The splenomegaly observed in this study was partly explained by the extramedullary granulocytosis but the spleen and other organs also showed proliferation of reticuloendothelial cells. One large study[9] and a smaller one[10] have both reported an enhanced incidence of leukaemia in mice after UVB irradiation. Together with the fact that the only leukaemia in our study occurred in a non-protected UV-irradiated mouse, this suggests that some of the lesions as found on histology may be of importance as to the development of leukaemia. However, reticulosis as defined here is a non-specific reaction which has also been reported in mice exposed to chemicals[24] and chronic antigenic stimulation.[25] The possibility that systemic responses, as seen in the mouse, may also be induced by UV-exposure in humans has not been studied.

Our study included a preliminary investigation of the effect of topical PABA. UVB irradiation together with PABA has been shown to be mutagenic *in vitro*,[26,27] and as PABA acts as a hapten it may cause photocontact and contact dermatitis.[28] Its protective effect with respect to UV-induced erythema and skin tumour development is well documented.[17] We found that PABA gave protection against UV-induced peripheral granulocytosis, reticulosis and splenomegaly. It is possible that these responses are contingent upon the development of skin inflammation. When applied to the skin of our mice PABA was absorbed into the general circulation (H.Flindt-Hansen, unpublished data) and it is unknown at present whether the protection is partly explained by its absorption; our finding of a reduced spleen weight in totally protected animals suggests that the compound has a systemic toxic effect.

Acknowledgments

We thank Professor Per Thune for the opportunity to carry out the study; the Institute of Medical Statistics, Ullevaal Hospital, for statistical assistance; Mr Stein Waagene for his assistance with the animals and the DAB Foundation for financial support.

References

1 Blum HF. *Carcinogenesis by Ultraviolet Light*. New York: Princeton University Press, 1959.

2 Stenbach F. Life history and histopathology of ultraviolet induced skin tumours. *Natl Cancer Inst Monogr* 1978; **50:** 57–70.

3 Kripke ML. Immunology and photocarcinogenesis. *J Am Acad Dermatol* 1986; **14:** 149–55.

4 Schacter B, Ledermann MM, Levine MJ, Ellner JJ. Ultraviolet radiation inhibits human natural killer activity and lymphocyte proliferation. *J Immunol* 1983; **130:** 2484.

5 Ledermann MM, Schacter B, LeVine MJ, Ellner JJ. Inhibition of human lymphocyte proliferation by ultraviolet radiation: Effects of ultraviolet-B (290 to 320 nm) on T-lymphocytes, monocyte accessory function, and induction of suppressor mechanisms. *J Lab Clin Med* 1986; **107:** 66–72.

6 Tamaki K, Stingl G, Katz SI. The origin of Langerhans cells. *J Invest Dermatol* 1980; **74:** 309–11.

7 Aberer W, Schuler G, Stingl G *et al.* Ultraviolet light depletes surface markers of Langerhans cells. *J Invest Dermatol* 1981; **76:** 202–10.

8 Orita M. Effects of ultraviolet irradiation on surface marker expression by epidermal immunocompetent cells and contact sensitization to dinitrofluorobenzene in mice. *Br J Dermatol* 1987; **117:** 721–33.

9 Ebbesen P. Enhanced lymphoma incidence in BALB/c mice after ultraviolet light treatment. *J Natl Cancer Inst* 1981; **67:** 1077–8.

10 Eaton GJ, Custer RP, Crane AR. Effects of ultraviolet light on nude mice. Cutaneous carcinogenesis and possible leucemogenesis. *Cancer* 1978; **42:** 182–8.

11 Morrison WL, Parrish JA, Block KJ, Krugler JI. *In vivo* effect of UVB on lymphocyte function. *Br J Dermatol* 1979; **101:** 513.

12 Hersey P, Haran G, Hasic E, Edwards A. Alterations of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. *J Immunol* 1983; **131:** 171–4.

13 Spode E. Untersuchungen über die Strahlenreaktion des Blutes. Veranderungen des peripheren Blutbildes nach UV-bestrahlung. *Strahlentherapie* 1956; **99:** 482.

14 Everett MA, Yeargers E, Sayre RM, Olsen RE. Penetration of epidermis by ultraviolet rays. *Photochem Photobiol* 1966; **5:** 533–42.

15 Bissett DL, Hannon PD, Orr TV. An animal model of solar aged skin: Histological, physical and visible changes in UV-irradiated hairless mice skin. *Photochem Photobiol* 1987; **46:** 367–78.

16 Johnston KJ, Oikarinen AL, Lowe NJ *et al.* Ultraviolet radiation-induced connective tissue changes in the skin of hairless mice. *J Invest Dermatol* 1984; **82:** 587–90.

17 Flindt-Hansen H, Thune P, Larsen TE. The inhibiting effect of PABA on photocarcinogenesis. *Arch Dermatol Res* 1990; **282:** 38–41.

18 Kendal M, Stuart A. *The Advanced Theory of Statistics*. London: Charles Griffin, 1979.

19 Lehmann EL. *Nonparametrics: Statistical Methods Based on Ranks*. San Francisco: Holden Day Inc., 1975.

20 Wester U. Solar ultraviolet radiation on the Canary Islands and in Sweden—a comparison of irradiance level. In: *Human Exposure to Ultraviolet Radiation: Risks and Regulations* (Passhier WF, Bosnjakovic BFM, eds). Amsterdam: Elsevier, 1987: 275–9.

21 Dunn TB. Normal and pathological anatomy of the reticular tissue in laboratory mice. *J Natl Cancer Inst* 1954; **14:** 1281–1433.

22 Dunn TB, Deringer MK. Reticulum cell neoplasm, type B, or the 'Hodgkin's-like lesion' of the mouse. *J Natl Cancer Inst* 1968; **40:** 771–821.

23 Hawk JLM, Murphy GM, Holden CA. The presence of neutrophils in human cutaneous ultraviolet-B inflammation. *Br J Dermatol* 1988; **118:** 27–30.

24 Laerum OD, Iversen OH. Reticuloses and epidermal tumours in hairless mice after topical application of cantharidin and asiaticoside. *Cancer Res* 1972; **32:** 463–9.

25 Metcalf D. Reticular tumours in mice subjected to prolonged antigenic stimulation. *Br J Cancer* 1961; **15**: 769–9.

26 Hodges NDM, Moss SH, Davies DJG. The sensitizing effect of a sunscreening agent, *p*-aminobenzoic acid, on near UV-induced damage in a repair deficient strain of *Escherichia coli*. *Photochem Photobiol* 1977; **26**: 493–8.

27 Sutherland JC, Griffin KP. *p*-Aminobenzoic acid can sensitize the formation of pyrimidine dimers in DNA: direct chemical evidence. *Photochem Photobiol* 1984; **40**: 391–4.

28 Thune P. Contact and photocontact dermatitis to sunscreens. *Photodermatol* 1984; **1**: 5–9

British Journal of Dermatology (1991) **125**, 227–232. ADONIS 000709639100206I

IgE antibodies to *Pityrosporum ovale* in atopic dermatitis

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Accepted for publication 15 April 1991

Summary An enzyme-linked immunosorbent assay (ELISA) was developed to assess serum IgE antibodies directed against *Pityrosporum ovale* in patients with atopic dermatitis (AD), atopic patients with allergic respiratory disease (ARD: rhinitis or asthma) but without eczema, and in healthy controls. IgE binding to *P. ovale* extract was demonstrated in 49% (35/72) of AD patients. In contrast, anti-*P. ovale* IgE was found in only one of 27 atopic controls without eczema; all healthy control sera ($n = 17$) were negative. Of 37 AD patients tested intracutaneously with *P. ovale*, 31 showed immediate-type reactivity, and 20 of these 31 patients had anti-*P. ovale* IgE detectable by ELISA, while sera from the six non-responders were all negative. Levels of anti-*P. ovale* IgE were highest in AD patients aged 20–30 years. No correlation was found with the severity of AD, but there was a non-significant tendency ($P = 0{\cdot}06$) to higher levels in AD patients with concomittant respiratory allergy. Anti-*P. ovale* IgE was significantly correlated with total serum IgE, with specific IgE against various aeroallergens as measured by RAST, and with levels of anti-*Candida albicans* IgE, measured with a similar ELISA. Thus, production of IgE antibodies against *P. ovale* occurs very frequently in AD, and rarely in patients with atopic disease without skin involvement.

Atopic dermatitis (AD) is associated with high levels of total and allergen-specific serum IgE and immediate-type reactions to a wide variety of allergens.[1,2] The aetiological role of allergic reactions has been demonstrated by the induction of inflammatory eczematous reactions after epicutaneous application of mite or pollen allergens on the apparently non-involved skin of AD patients.[3-6] Although the histopathology and kinetics of these lesions and of the 'spontaneously' involved skin in AD indicate a delayed-type reaction, it is probable that allergen-specific IgE is involved.[7-10] Atopic patients with allergic respiratory disease (ARD) but no eczema do not, or only occasionally, respond to patch tests with aeroallergens, in spite of frequently enhanced levels of anti-mite or anti-pollen IgE.[3,4] Thus, skin reactivity towards IgE-binding allergens that enter percutaneously may be a characteristic feature of AD.

This proposed pathogenic mechanism points to the potential significance of allergens derived from the normal skin microflora, in addition to airborne mite and pollen allergens. The presence of such allergens might constitute an important permanent stimulus for allergic skin reactions. One source of possibly relevant allergens is the lipophilic yeast *Pityrosporum ovale* which can be isolated from the skin of up to 90% of healthy adults, especially from sebaceous areas of the face, scalp, upper back and chest.[11,12] Although normally non-pathogenic, *P. ovale* has been associated with skin diseases such as pityriasis versicolor, folliculitis, seborrhoeic eczema and psoriasis.[13-15] Clemmensen and Hjorth[16] showed that treatment with oral ketoconazole improved the skin lesions in a subgroup of AD patients with predominant involvement of the head and neck region and type I skin reactions to *P. ovale*. Recently, immediate and delayed-type anti-*P. ovale* skin reactions have been described in large series of AD patients.[17-20] These reactions might substantially contribute to the pathogenesis of AD lesions, and assessment of anti-*P. ovale* IgE antibodies may be of diagnostic value. We have therefore developed a sensitive enzyme-linked immunosorbent assay (ELISA), which was used to compare levels of anti-*P. ovale* IgE in the sera of AD patients and of atopic controls with ARD but no eczema. The results confirm the frequent and specific occurrence in AD of IgE-mediated hypersensitivity to *P. ovale*.

Methods

Patients

Sera were collected from 103 atopic patients attending the Utrecht University Hospital between September 1988 and June 1990. Seventy-two patients had AD (56

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women, 20 men; median age 23·9 years, range 2–52 years) and 27 had atopic respiratory disease (ARD; asthma or rhinitis) without eczema (15 women, 12 men; median age 32·6 years, range 8–63 years). Four sera were included from atopic adults with current ARD, who had suffered from eczema in their childhood. Control sera ($n=17$) were obtained from healthy laboratory and hospital staff. All sera were stored at −70°C until use.

Only patients with atopic disease confirmed by *in-vitro* diagnostic tests (total serum IgE > 150 IU/ml and/or a positive RAST to one or more aeroallergens) were included in both the AD and ARD group. All AD patients fulfilled the Hanifin and Rajka criteria for AD.[21] Fifty-three AD patients had a personal history of associated ARD and in 37 of these a family history of ARD was also noted. In 13 of the other 19 AD patients there was a known family history of atopic disease. Sera from 35 patients was obtained during hospitalization because of an exacerbation of their AD.

Most patients had been tested intracutaneously with several allergens: grass pollen (100 AU/ml), tree pollen (100 AU/ml), mould-mix (0·1%; *Aspergillus fumigatus Penicillium notatum, Alternaria alternata, Cladosporium cladosporioides, Mucor mucedo*), house dust (0·5%), *Dermatophagoides pteronyssinus* (10 AU/ml), *Artemisia* pollen (10 AU/ml), cat and dog dander (0·01%), and human dander (0·1%) (all from HAL Allergenen Laboratorium, Haarlem, The Netherlands). Thirty-seven AD patients had been tested intracutaneously with a 1:100 diluted *P. ovale* extract prepared for skin prick tests by ALK Laboratories, Copenhagen, Denmark (Soluprick, 5 mg/ml in 50% glycerol, lot no. 015/83115101).[17]

IgE determinations

Total serum IgE was measured by PRIST (Pharmacia) and specific IgE levels to atopic allergens such as house dust, house dust mite, several pollen extracts, and cat and dog dander by RAST (Pharmacia). Twenty-nine of the AD sera had also been tested by a RAST for anti-*P. ovale* IgE.[17]

ELISA for anti-P. ovale IgE

The *P. ovale* extract from ALK (Soluprick, 5 mg/ml; lot no. 015/83115101) was also used in the ELISA for anti-*P. ovale* IgE. The extract was dialysed overnight at 4°C against phosphate-buffered saline (PBS, pH 7·4) to remove the glycerol, and stored in aliquots at −40°C. Microtitre plates (Maxisorp, no. 4-39454A; Nunc, Roskilde, Denmark) were coated with a 1:50 dilution of the extract in 0·1 M carbonate/bicarbonate buffer, pH 9·5. In each step of the assay (except the last) 0·1 ml portions were added to the wells. Coating was performed over night at 37°C. The wells were rinsed extensively with PBS containing 0·05% Tween-20 (PBS-Tween). Serum samples diluted 1/10 in PBS-Tween were added, and incubated with shaking for 2 h at room temperature. Subsequent incubations took place for 1 h at 37°C or (with continuous shaking) at room temperature, and were followed by extensive washing with PBS-Tween. Binding of IgE was assessed by incubation with mouse monoclonal anti-human IgE (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands, batch no. MH25-01-MO2), diluted 1/3000 in PBS-Tween containing 1% gelatin and 0·1 mg/ml azide, followed by incubation with biotinylated rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark), diluted 1/1000 in PBS-Tween, and thereafter with avidin-peroxidase (Dakopatts) at 1/2000 in PBS-Tween. Finally, each well received 0·15 ml of a solution of 2·1 mM *o*-phenylenediamine (OPD; Sigma Chemicals, St Louis, MO, U.S.A.) and 4·4 mM H_2O_2 in 33 mM phosphate-citrate buffer, pH 4·9. After 30 min at room temperature, the reaction was stopped with 0·05 ml 2·5 N H_2SO_4, and the absorption was read at 490 nm. Each sample was tested in duplicate, in both *P. ovale*-coated and in non-coated control wells. All data were corrected for OD(490) values in non-coated wells, which were in the range of 0·05–0·20. In each microtitre plate at least two PBS controls in coated and non-coated wells were tested to exclude any anti-*P. ovale* reactivity of the various other reagents. OD(490) values in such PBS-treated wells were low (<0·1) and did not differ between coated and non-coated wells. A positive AD serum giving at a 1/10 dilution a corrected OD(490) value of 0·8–1·0, was included in each plate as a positive control.

Anti-*Candida* IgE was determined in an identical ELISA procedure, with microtitre plates coated with *Candida albicans* extract (Laboratorium Diephuis, Groningen, The Netherlands) at 0·1 mg/ml.

Results

Presence of anti-P. ovale serum IgE

The ELISA results of patients and controls are presented in Figure 1. Positivity was defined as an OD(490) exceeding the mean OD(490) + 3 SD of the healthy control group, which meant a value > 0·11. Thus, 35 of the 72 AD patients (49%) had detectable anti-*P. ovale* IgE antibodies. In contrast, only one of the sera of 27

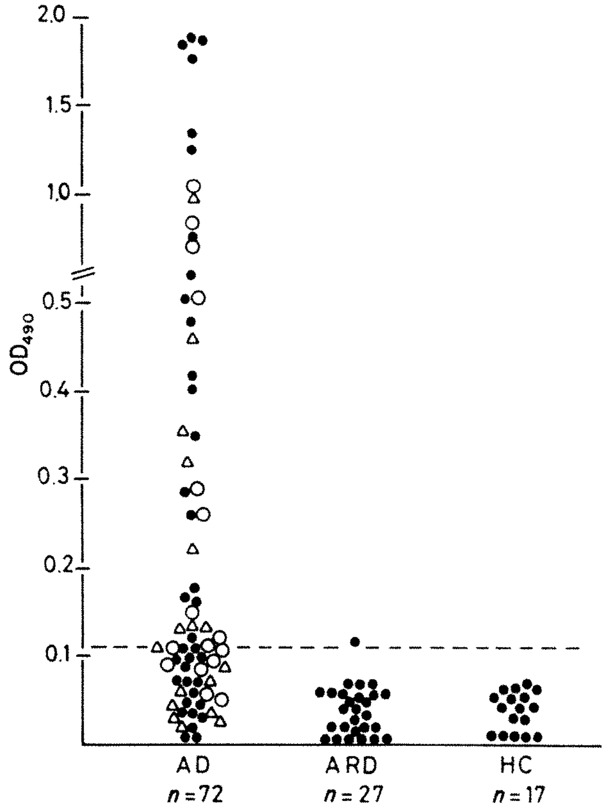

Figure 1. Anti-*P. ovale* IgE antibodies determined by ELISA in 1/10 diluted sera from 72 patients with atopic dermatitis (AD), 27 patients with atopic rhinitis or asthma, but no eczema (ARD), and 17 healthy controls (HC). AD patients had either an individual history of associated ARD (○), only a family history of atopic disease (△), or both (●). OD(490) values were all corrected for non-specific binding of serum IgE in non-coated and of test reagents in coated wells.

patients with ARD and no skin involvement was found to be positive. The sera of four atopic controls with present ARD and previous eczema in their childhood were all negative (data not shown). When the mean OD(490) + 2 SD of healthy control sera was used as the cut-off value (0·85), the sensitivity of the assay for AD increased to 68% (49/72), and again only one of the ARD sera was positive. In further analyses, the mean OD(490) + 3 SD of healthy control sera was used as the detection limit.

Correlation with RAST data

The sera of 29 AD patients had been tested previously in a RAST for anti-*P. ovale* IgE.[17] Concordant results were found in 79% (23/29; 10 negative, 13 positive); four sera were ELISA −/RAST + and two sera ELISA +/RAST −. There was also a good quantitative correlation between OD(490) values in the ELISA and percentage binding of radiolabelled anti-IgE in the RAST ($r=0{\cdot}74$; Spearman rank correlation).

Intracutaneous tests with P. ovale

Of 37 AD patients tested intracutaneously (i.c.) with *P. ovale* extract, 31 (84%) showed immediate-type reactivity. Twenty of the 31 responders, but none of the six patients with a negative i.c. test, had detectable anti-*P. ovale* IgE. Thus a good correlation was demonstrated between the ELISA and the i.c. test ($P=0{\cdot}005$, Fisher's exact test), although the ELISA appeared to be less sensitive. No correlation was found between the intensity of the i.c. reactions and OD(490) values in the ELISA.

Age and anti-P. ovale IgE levels

Because the occurrence of *P. ovale* on the skin depends on age,[22,23] the relation of the IgE anti-*P. ovale* levels to the age of the AD patients was studied. The highest concentrations were found among patients aged 20–30 years (Fig. 2). Although the majority (45/72) of the AD patients belonged to this age category, there was a significant predominance of moderate and high levels of anti-*P. ovale* IgE in this decade, compared to the combined other age categories ($P<0{\cdot}01$; Wilcoxon's rank order sum test).

Associated ARD and severity of eczema

No significant difference was found between the ELISA results in patients being treated as out-patients (17/37 positive; median OD(490) value 0·11) and those hospitalized with an exacerbation of AD and, in most cases, involvement of a large proportion of the whole body area

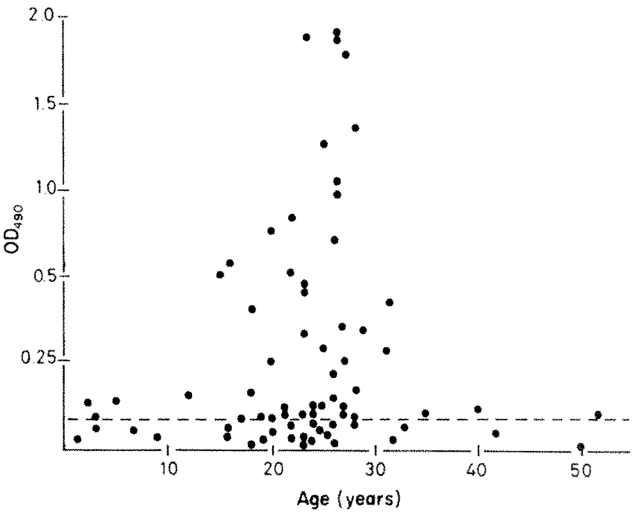

Figure 2. Anti-*P. ovale* IgE levels in AD patients as a function of age.

Table 1. Correlations between serum levels of anti-*Pityrosporum ovale* IgE (ELISA) and levels of total (PRIST) and specific (RAST) IgE in AD patients

Allergen	*n**	*r*†	*P* value
Dust mite (*D. pteronyssinus*)	60	0·38	<0·001
Tree pollen	59	0·27	<0·02
Artemisia pollen	42	0·59	<0·0001
Grass pollen	59	0·16	<0·1
Dog dander	17	0·38	<0·02
Cat dander	17	0·45	<0·04
Candida albicans‡	54	0·74	<0·0001
Total IgE	65	0·74	<0·0001

* Number of patients for whom data of both tests were available.
† Spearman's rank correlation coefficient.
‡ Determined by ELISA.

(18/35 positive; median OD(490) value 0·12). Comparison of AD patients with or without a personal history of respiratory allergy revealed a non-significant tendency ($P = 0{\cdot}06$; Wilcoxon) to higher levels of anti-*P. ovale* IgE in AD with associated present or previous ARD (Fig. 1).

Other parameters of atopy

Within the AD group the results (positive/negative) of i.c. tests with *P. ovale* and with various other allergens were compared. Significant correlations (Fisher's exact test) were found with i.c. tests with human dander and with grass pollen, but not with other allergens (data not shown). When the results of the anti-*P. ovale* ELISA were compared with the levels of total and specific IgE as determined by PRIST and RAST, significant correlations were found with total serum IgE, and with specific IgE against house dust, house dust mite, tree pollen, *Artemisia* pollen, and dog and cat dander (Table 1). No correlation was found with RAST results for grass pollen. Of 54 AD sera tested in an identical ELISA procedure with a coating of *Candida* instead of *Pityrosporum* extract, 14 gave an OD(490) value >0·11. Thirteen of these 14 sera also had detectable anti-*P. ovale* IgE, and a highly significant correlation between the levels of both types of IgE was found (Table 1). As with anti-*P. ovale* IgE, anti-*Candida* IgE was only found in AD, and not in ARD patients without eczema (data not shown).

Discussion

Several recent reports have described a high frequency of immediate and/or delayed type anti-*Pityrosporum* reactivity in patients with AD. Rokugo *et al.*[18] reported delayed-type reactions to scratch-patch tests in 64% of AD patients, and positive skin prick tests (SPT) in 54%[18] but no data was given as regards anti-*P. ovale* IgE. Kieffer *et al.*[19] found a similar high frequency of positive SPT (65%), and *P. ovale*-specific IgE was demonstrated in 64%, using a basophil histamine release assay, which correlated well with the SPT results.[19] Nordvall and Johansson[20] assessed anti-*P. ovale* IgE in 105 sera from atopic children with or without eczema. Their results agreed with our previous and present findings in that a positive RAST was found mainly in patients with AD or AD+ARD (38%), and only incidentally (6%) in patients with ARD alone. In our previous study,[17] the majority of adult AD patients reacted positively to intracutaneous tests (87%) or SPT (59%) with *P. ovale* extracts. None of the atopic controls with ARD but no eczema responded to SPT and only 12% reacted to i.c. tests.[17] RAST testing was performed with a culture filtrate of *P. ovale*, and binding of IgE was found in 65% of AD sera but was virtually absent from the sera of atopic controls without skin disease.

Because we preferred a non-radioactive immunoassay, a sensitive ELISA was developed with microtitre plates coated with *P. ovale* extract. Only small amounts of serum (less than 0·05 ml) were required for duplicate measurements in coated and in non-coated control wells. The assay can be easily performed with the same allergen solution as used for *in vivo* diagnostic tests. Although we used partial denaturation in alkaline coating buffer in the present study the ELISA can also be performed with *P. ovale* extract coated in PBS (unpublished data). Despite the technical differences, the ELISA results correlated well with the previously used RAST method, with only 20% (6/31) of AD sera giving discordant results; five of these six sera had OD(490) values and binding percentages near the lower detection limits of both assays.

Our results may have been influenced by the relatively large proportion of severe cases of AD, as nearly 50% of the sera were obtained from hospitalized patients with exacerbating AD. No difference was found, however, between the levels of anti-*P. ovale* IgE in the out-patients and in-patients with atopic dermatitis suggesting no relationship with the severity of the disease.

Our data confirm the previously reported specificity of anti-*P. ovale* IgE for AD. The marked correlations with total and specific serum IgE suggest that atopy with a predisposition to produce IgE antibodies is necessary for the production of anti-*P. ovale* IgE, but its association with AD indicates that other factors are also involved. The presence of IgE against skin microflora might be one of the aetiological factors that distinguish AD patients from atopics without skin disease. Alternatively, the occurrence of anti-*P. ovale* IgE might be a secondary phenomenon, due to an increased exposure to the yeast allergens. Its relation to age suggests that the skin colonization rate is of importance, because the highest anti-*P. ovale* IgE levels were found in patients aged 20–30 years, and *P. ovale* colonization in healthy controls reaches its maximum in the third decade of life.[22,23] Preliminary studies have shown an increased colonization with *P. ovale* in lesional, compared to the healthy skin in these patients (J.E.M.H.van Bronswijk and E.Young, unpublished data). It is not known whether lesions induced by scratching may provide a suitable environment for the enhanced growth of *P. ovale*. However, even if increased exposure to *P. ovale*-derived allergens is a secondary phenomenon, the subsequent IgE-mediated skin reactions to *P. ovale*-derived allergens may contribute significantly to the severity of exacerbations. The beneficial effect of oral anti-mycotic treatment[16,24] is suggestive for a causative role for *P. ovale* in some forms of AD. Trials are currently being carried out with topical ketoconazole to determine if this is an effective treatment.

The correlation between levels of anti-*P. ovale* IgE and levels of IgE binding to *Candida* allergens agrees with the data reported by Nordvall and Johansson.[20] Anti-*Candida* immediate-type reactivity has been suggested to be associated with bronchial asthma.[25-27] However, so far we have found anti-*Candida* IgE, like anti-*P. ovale* IgE, only in AD, and not in respiratory allergy without eczema. A more extensive study with a larger series of asthma patients should reveal whether anti-*Candida* IgE only occurs in asthmatics who also have eczema. Preliminary inhibition ELISA experiments have indicated that anti-*Candida* IgE in a number of AD patients is completely cross-reactive with extracts of *P. ovale* (G.Doekes, unpublished data). This would suggest that type I immunoreactivity to *P. ovale* might result from sensitization to *Candida* species. On the other hand, anti-*P. ovale* sensitization might strongly influence the immunoreactivity of AD patients towards other commensal or infective micro-organisms like *C. albicans*, e.g. in secondarily infected skin lesions. Whatever the origin of the IgE-mediated reactions with common microbial antigens, their role in AD may be significant.

References

1 Juhlin L, Johansson SGO, Bennich H *et al.* Immunoglobulin E in dermatoses. *Arch Dermatol* 1969; **100**: 12–16.

2 Mackie R, Cobb S, Cochrane R, Thomson J. Total and specific IgE levels in patients with atopic dermatitis. *Clin Exp Dermatol* 1979; **4**: 187–95.

3 Mitchell E, Chapman M, Pope F *et al.* Basophils in allergen-induced patch test sites in atopic dermatitis. *Lancet* 1982; i: 127–31.

4 Reitamo S, Visa K, Kahonen K *et al.* Eczematous reactions in atopic patients caused by epicutanous testing with inhalent allergens. *Br J Dermatol* 1986; **114**: 303–9.

5 Gondo A, Saeki N, Tokudo Y. Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen. *Br J Dermatol* 1986; **115**: 485–92.

6 Bruijnzeel-Koomen CAFM, van Wichen DF, Spry CJF *et al.* Active participation of eosinophils in patch test reactions to inhalent allergens in patients with atopic dermatitis. *Br J Dermatol* 1988; **118**: 229–38.

7 Bruijnzeel-Koomen CAFM, van Wichen DF, Toonstra J *et al.* The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch Dermatol Res* 1986; **278**: 199–205.

8 Bruijnzeel-Koomen CAFM, Bruijnzeel PLB. A role for IgE in patch test reactions to inhalant allergens in atopic dermatitis. *Allergy* 1988; **43**: (Suppl. 5): 15–21.

9 Barker JNWN, Alegre VA, MacDonald DM. Surface-bound immunoglobulin E on antigen-presenting cells in cutaneous tissue of atopic dermatitis. *J Invest Dermatol* 1988; **90**: 117–21.

10 Bieber T, Dannenberg B, Prinz JC *et al.* Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the serum IgE level. *J Invest Dermatol* 1989; **93**: 215–19.

11 Roberts SOB. *Pityrosporon orbiculare*: Incidence and distribution on clinically normal skin. *Br J Dermatol* 1969; **81**: 264–9.

12 Faergemann J, Aly R, Maibach HI. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. *Acta Derm Venereol (Stockh)* 1983; **63**: 346–8.

13 Back O, Feargemann J, Hornquist R. *Pityrosporon* folliculitis: A common disease of the young and middle aged. *J Am Acad Dermatol* 1985; **12**: 56–61.

14 Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. *Br J Dermatol* 1984; **111**: 235–42.

15 Rosenberg EW, Belew PW. Improvement of psoriasis of the scalp with ketoconazole. *Arch Dermatol* 1982; **118**: 370–1.

16 Clemmensen OJ, Hjorth N. Treatment of dermatitis of the head and neck with ketaconazole in patients with type I sensitivity to *Pityrosporon orbiculare*. *Semin Dermatol* 1983; **2**: 26–9.

17 Young E, Koers WJ, Berrens L. Intracutaneous tests with *Pityrosporon* extract in atopic dermatitis. *Acta Derm Venereol (Stockh)* 1989; Suppl. **144**: 122–4.

18 Rokugo M, Tagami H, Usuba Y, Tomita Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. *Arch Dermatol* 1990; **126**: 627–32.

19 Kieffer M, Bergbrant I-M, Feargemann J *et al.* Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. *J Am Acad Dermatol* 1990; **22**: 739–42.

20 Nordvall SL, Johansson S. IgE antibodies to *Pityrosporum ovale* in children with atopic diseases. *Acta Paediatr Scand* 1990; **79**: 343–8.

21 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; Suppl. **92**: 44–7.

22 Faergemann J, Frederiksson T. Age incidence of *Pityrosporum orbiculare* on human skin. *Acta Derm Venereol (Stockh)* 1980; **60**: 531–3.

23 Bergbrant I-M, Faergemann J. Variations of *Pityrosporum orbiculare* in middle-aged and elderly individuals. *Acta Derm Venereol (Stockh)* 1988; **68**: 537–40.

24 Wearstedt A, Hjorth N. *Pityrosporon orbiculare*—a pathogenic factor in atopic dermatitis of the face, scalp and neck? *Acta Derm Venereol (Stockh)* 1985; Suppl. 114: 146–8.

25 Edge G, Pepys J. Antibodies in different immunoglobulin classes to *Candida albicans* in allergic respiratory disease. *Clin Allergy* 1980; 10: 47–58.

26 Gumowski P, Lech B, Chaves I, Girard J-P. Chronic asthma and rhinitis due to *Candida albicans*, epidermophyton, and trichophyton. *Ann Allergy* 1987, 59: 48–51.

27 Koivikko A, Kalimo K, Nieminen E, Viander M. Relationship of immediate and delayed hypersensitivity to nasopharyngeal and intestinal growth of *Candida albicans* in allergic subjects. *Allergy* 1988; 43: 201–5

British Journal of Dermatology (1991) **125**, 233–237.

ADONIS 000709639100207V

Ultrastructural localization of pemphigus vulgaris and pemphigus foliaceus antigens in cultured human squamous carcinoma cells

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Accepted for publication 26 April 1991

Summary The ultrastructural localization of the pemphigus vulgaris (PV) and pemphigus foliaceus (PF) antigens in cultured human squamous carcinoma cells was observed using immunogold electron microscopy. Both the PV and PF autoantibodies bound only to the extracellular portion of the desmosomal structures. After incubation at 37°C, the PV antigen–antibody complexes were observed within the cultured cells. PV and PF antigen expression was markedly reduced when the cells were cultured in medium with a low Ca^{2+} concentration.

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases in which there are circulating autoantibodies to the cell surface of the keratinocytes. Recently, biochemical studies using immunoprecipitation and immunoblotting have identified the PV antigen as a 130-kDa glycoprotein.[1–4] This protein is present as a 210-kDa protein complex with a 85-kDa protein, plakoglobin, which is a cytoplasmic structural component present in both desmosomes and adherens junctions of the cell surface.[2,3,5] PF sera bind to a 160-kDa protein that is now thought to be desmoglein, a transmembranous desmosomal glycoprotein.[2,4,6–8] Assembly of the 160-kDa PF antigen and plakoglobin also forms a protein complex of 260 kDa.[3,5,9] These data suggest that the PV antigen may be present in both desmosomes and/or adherens junctions, whereas the PF antigen may be localized in desmosomal areas of the keratinocyte cell membrane.[8] However, there is controversy over the ultrastructural localization of the PV and PF antigens. Some studies showed the presence of pemphigus antigen throughout the keratinocyte plasma membrane,[10–12] but localization only to the desmosomes has also been reported.[13] In the present study, we investigated the ultrastructural localization of the PV and PF antigens in cultured human keratinocytes using immunogold electron microscopy. Our study was also designed to clarify the fate of the antigen–antibody complexes and the influence of a low concentration of Ca^{2+} in the culture medium on the expression of the PV and PF antigens.

Methods

Cells

In this study, we used the KU-8 cell line[14] (a gift from the Department of Urology, Keio University School of Medicine), which had been established from a lymph-node metastasis of human squamous cell carcinoma of the penis. A previous electron microscopic and immunohistochemical study confirmed that these cultured KU-8 cells form desmosomes.[14] The cells were cultured in DMEM (Gibco Laboratories, Grand Island, NY, U.S.A.) supplemented with 10% foetal calf serum (FCS) (Gibco Laboratories) and penicillin/streptomycin (Gibco Laboratories) (final Ca^{2+} concentration: approx. 1·2 mM) on Lab-Tek tissue culture chamber slide (Nunc Inc, Naperville, IL, U.S.A.).

Pemphigus sera

Sera from four patients with PV and three patients with PF who presented typical clinical, histological and immunohistological features were used in this study. Two sera from normal volunteers were used as controls. Immunoblot studies using normal human epidermal extract as an antigen source showed that all PV and PF sera reacted to the 130-kDa antigen and the 150-kDa PF antigen (identical to the 160-kDa desmoglein), respectively.[4]

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Immunostaining methods

To demonstrate the ultrastructural localization of the antigens, we used immunoelectron microscopy with gold colloid particles.[15] After pre-incubation in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA: Sigma Chemical Co., St Louis, MO, U.S.A.) for 30 min, KU-8 cells were incubated in the PV or PF sera, diluted 1 : 10 in PBS/1% BSA, in the presence of 0·1% NaN$_3$ at 4°C for 2 h.[1] After three washes in PBS/1% BSA, the cells were incubated with 5 nm gold-conjugated goat anti-human IgG (E-Y Laboratories, San Mateo, CA, U.S.A.), diluted 1 : 10 in PBS/1% BSA, at 4°C for 1 h with NaN$_3$. After careful washing with PBS, the cells were fixed with 2% glutaraldehyde, post-fixed with 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a JEM-1200 EX II electron microscope.

To demonstrate the fate of the antigen–antibody complex, the cells were incubated with the sera at 4°C for 2 h without NaN$_3$, and subsequently with the second antibody at 37°C for 45 min or 2 h without NaN$_3$.

To examine the effects of Ca^{2+} concentration in the culture medium, cells were cultured in the low Ca^{2+} medium, Ca^{2+}-free MEM (Gibco Laboratories) with 5% FCS with a final Ca^{2+} concentration of approx. 0·2 mM[16] for 24 h and used for the immunolabelling as described above.

Results

Ultrastructural binding sites of the PV and PF antibodies

Cultured KU-8 cells showed many cytoplasmic projections which formed with other cells desmosomal structures (Fig. 1). These structures were sometimes also observed at points where there were no apparent cell-to-cell contacts.

In the cells labelled with the PV sera, similar results were obtained with all four sera. Gold colloid particles bound only to the desmosomal structures and interdesmosomal areas were not labelled. Gold deposition was observed on the desmosomal structures on the flat cell surface and at the surface of thin cytoplasmic projections (Fig. 2a). The particles were densely deposited on the extracellular portion of the desmosomal structure (Fig. 2b).

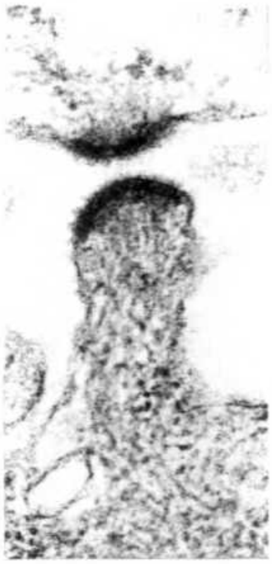

Figure 1. Desmosomal structure where a cytoplasmic projection of a cultured KU-8 cell is attached to the surface of an adjacent cell (standard fixation without immunolabelling, × 90,000).

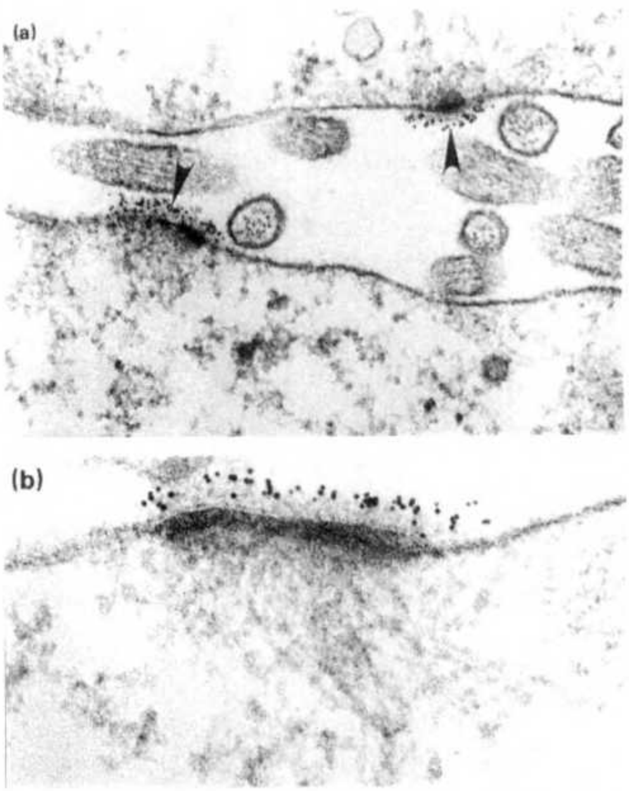

Figure 2. (a) Localization of PV antibody to desmosomal structures (arrowheads). No gold particles are seen in the interdesmosomal area (× 59,500). (b) Gold particles on the extracellular portion of the desmosomal structure. Within the cell intermediate filaments insert into a dense plaque (× 11,400).

In the cells treated with the PF sera, the gold particles were observed only on the desmosomal structures. However, the deposition was limited to the desmosomal areas in the cytoplasmic projections or at their adjacent

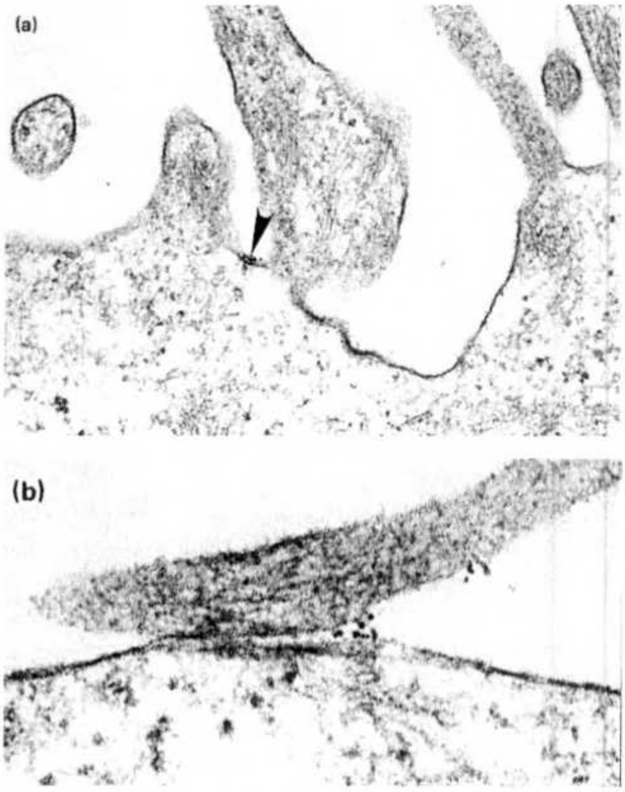

Figure 3. PF antibody stain. (a) Gold particles are observed on a desmosomal structure (arrowhead) (× 42,000). (b) Gold particles are seen on the extracellular portion of the desmosomal structure (× 80,750).

cell surface (Fig. 3a). Gold particles also bound to the extracellular portion of the desmosomal structures (Fig. 3b). These results were observed with all three PF sera.

Effect of Ca^{2+} concentration on PV and PF antigen expression

Cell-to-cell contacts and the formation of desmosomal structures were reduced in the cells cultured in the low Ca^{2+} medium.

Gold deposition was observed on the desmosomal structures in those cells cultured in the low Ca^{2+} medium, but the number of gold particles with both the PV and PF sera was markedly reduced. With each serum, counts were made along 1200 μm of the cell

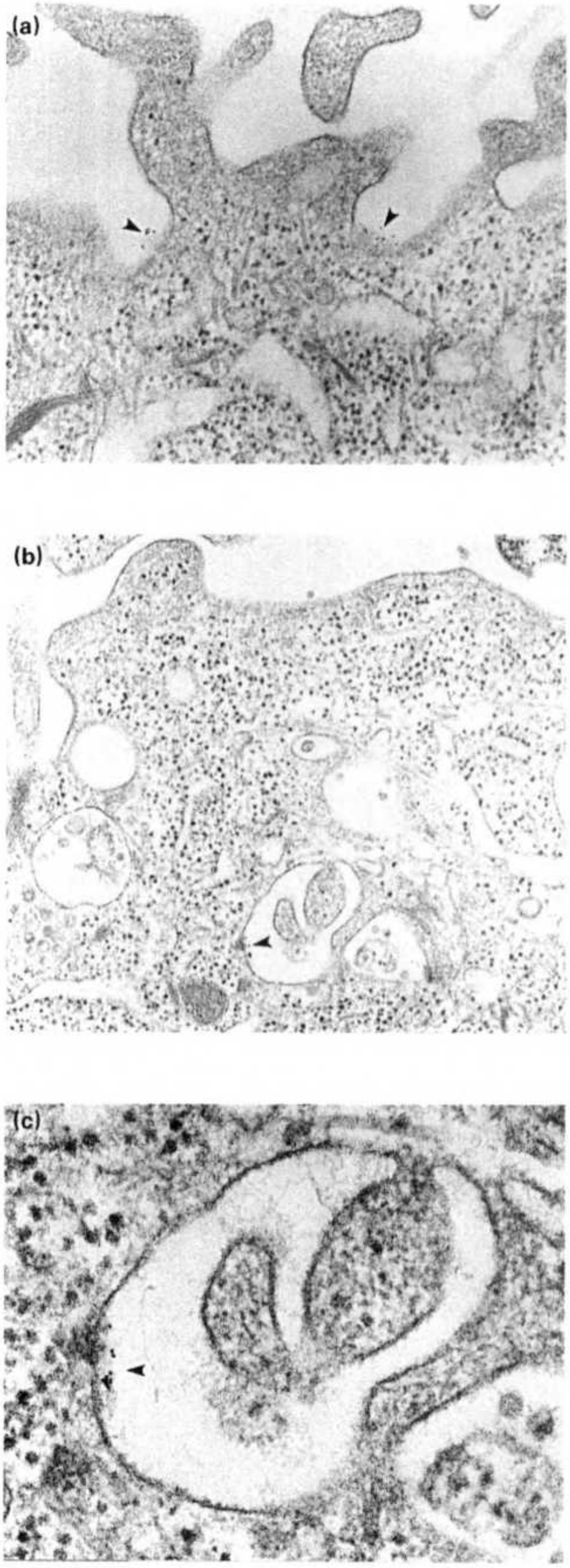

Figure 4. Internalization of antigen–antibody complexes. (a) After 45 min of incubation at 37°C, indentation of the cell surface is observed in the gold-labelled desmosomal area (arrowheads) (× 37,200). (b) After 2 h of incubation, a cytoplasmic vacuole that contains a gold-labelled desmosomal structure (arrowhead) is seen (× 27,900). (c) High-power view of Figure 4(b). The gold-labelled desmosomal structure is preserved in the cytoplasmic vacuole (arrowhead) (× 93,000).

surface of 30 cells. With PV sera, there were 1·02 ± 0·13 gold particles per 100 nm of cell surface in the cells cultured in the normal Ca^{2+} medium, and 0·072 ± 0·013 particles per 100 nm of cell surface in the cells cultured in the low Ca^{2+} medium. With PF sera, there were 0·36 ± 0·05 gold particles per 100 nm of cell surface in the cells cultured in the normal Ca^{2+} medium, and 0·021 ± 0·005 particles per 100 nm of cell surface in the cells cultured in the low Ca^{2+} medium.

Internalization of PV antigen–antibody complexes

After 45 min of incubation at 37°C with the second antibody, invagination of the plasma membrane was observed at the gold-labelled desmosomal areas (Fig. 4a). Gold particles were seen in cytoplasmic vacuoles after 2 h of incubation at 37°C. No antigen–antibody complex remained at the cell surface (Fig. 4b), but gold-labelled desmosomal structures in cytoplasmic vacuoles were identified (Fig. 4c).

Discussion

There is still controversy as to the ultrastructural localization of the pemphigus antigens. Previous immunoperoxidase and immunoferritin electron microscopic studies have indicated that the PV antibody binds to both desmosomes and the non-desmosomal areas of the cell membrane of keratinocytes.[10–12] However, increased binding of IgG to desmosomes compared to interdesmosomal areas has recently been demonstrated in the skin of patients with PV using immunoferritin labelling.[13] An immunofluorescence study of mouse keratinocytes also suggested that pemphigus antibody binds to desmosomes.[17,18] However, a study using monkey oesophagus and the results of ELISA coated with bovine desmosome did not demonstrate the binding of PV sera to desmosomes.[19]

In the present study, we employed immunogold electron microscopy to clearly demonstrate the antibody binding sites. In order to exclude the effects of fixation of the tissues, we used non-fixed cultured cells. We succeeded in demonstrating the binding sites of the PV and PF sera and our results indicate that in the cultured cells used, the PV and PF antigens were present only in the desmosomal areas. These results are consistent with those of a previous study of *in-vivo* bound IgG using immunoferritin labelling.[13] PV antigen has been identified as a 130-kDa glycoprotein,[1–4] whereas PF antigen is known to be a 160-kDa transmembranous desmosomal glycoprotein, desmoglein.[2,4,6–8,20] Our results confirm that some epitopes for the PV and PF autoantibodies are located on the extracellular domains of these desmosomal proteins. In the present study, the PF antigen was expressed in the desmosomal areas of the cytoplasmic projections or close to them. This pattern of expression suggests that the PF antigen is a more differentiated antigen, associated with cell–cell contact, than the PV antigen. However, the possibility cannot be excluded that there is a difference in the localization of the PV and PF antigens between cultured cells and normal human epidermis. It was suggested that the PV antigen may be present at the adherens junction[3] but as these junctions were not clearly seen in our culture system, we cannot provide further information as to the binding of PV and PF antibodies.

On electron microscopy, desmosomal structures were seen where there was no apparent cell-to-cell contact. One explanation may be that the three-dimensional nature of cell-to-cell contact means that it cannot be seen in some ultrathin sections. Another possibility is that KU-8 cells may have a different type of desmosome assembly from normal epidermal keratinocytes and may form immature desmosomal structures at the non-contact area.

Within 45 min after incubation, invagination of the plasma membrane occurred. Subsequently, cytoplasmic vacuoles containing the antigen–antibody complex were formed. In our study, internalization of the antigen–antibody complexes associated with gold particles was seen in PV. A similar phenomenon has been reported previously.[12,13,21] Kitajima *et al.*[22] suggested that the time required before cell–cell detachment occurs after the addition of PV sera to cultured squamous cell carcinoma cells might relate to the internalization of the antigen–antibody complex. More studies are required to determine whether this internalization plays an important pathogenetic role or represents only a non-specific endocytotic reaction.

In the present study, the expression of PV and PF antigens was much reduced in cells cultured in the low Ca^{2+} medium. A reduction in the expression of PV antigen on keratinocytes cultured in a low Ca^{2+} concentration has been reported using immunofluorescence[22,23] and on a haemadsorption assay.[24] We have confirmed the reduction of the expression of the PV and PF antigens using an immunogold method and electron microscopy. The presence of Ca^{2+} is known to enhance the detection by immunofluorescence of the PV antigen in tissue sections[25] and the presence of a Ca^{2+}-dependent epitope of the PF antigen was suggested.[9,26] However, it was reported that Ca^{2+} did not affect the transcription of the PV antigen mRNA, although Ca^{2+}-induced expression of PV antigen required new protein synthesis.[22] At present, it is unclear whether Ca^{2+} increases the reactivity of PV and PF antibodies by promoting antigen synthesis, by stabilizing the epitope conformation, or by some other mechanism.

To date, the localization of the pemphigus antigen in normal skin using immunogold electron microscopy has not been successful because of the difficulty of fixing tissue without losing the antigenicity. Further improvements in techniques are likely to overcome this problem.

Acknowledgments

This study was supported in part by a research grant from the Tokyo Metropolitan Hygiene Office and a grant from the Ministry of Health and Welfare, Japan.

References

1 Stanley JR, Yaar M, Hawley-Nelson P, Katz SI. Pemphigus antibodies identify a cell surface glycoportein synthesized by human and mouse keratinocytes. *J Clin Invest* 1982, **70:** 281–8.

2 Stanley JR, Koulu L, Thivolet C. Distinction between epidermal antigens binding pemphigus vulgaris and pemphigus foliaceus autoantibodies. *J Clin Invest* 1984; **74:** 313–20.

3 Korman NJ, Eyre RW, Klaus-Kovtun V, Stanley JR. Demonstration of an adhering-junction molecule (plakoglobin) in the autoantigens of pemphigus foliaceus and pemphigus vulgaris. *N Engl J Med* 1989, **321:** 631–5.

4 Hashimoto T, Ogawa MM, Konohana A, Nishikawa T. Detection of pemphigus vulgaris and pemphigus foliaceus antigens by immunoblot analysis using different antigen sources. *J Invest Dermatol* 1990; **94:** 327–31

5 Eyre RW, Stanley JR. Identification of pemphigus vulgaris antigen extracted from normal human epidermis and comparison with pemphigus foliaceus antigen. *J Clin Invest* 1988, **81:** 807–12.

6 Koulu L, Kusumi A, Steinberg MS *et al.* Human autoantibodies against a desmosomal core protein in pemphigus foliaceus. *J Exp Med* 1984; **160:** 1509–18

7 Stanley JR, Koulu L, Klaus-Kovtun V, Steinberg MS. A monoclonal antibody to the desmosomal glycoprotein desmoglein I binds the same polypeptide as human autoantibodies in pemphigus foliaceus. *J Immunol* 1986; **136:** 1227–30.

8 Ogawa MM, Hashimoto T, Konohana A *et al.* Immunoblot analyses of Brazilian pemphigus foliaceus antigen using different antigen sources. *Arch Dermatol Res* 1990; **282:** 84–8.

9 Eyre RW, Stanley JR Human autoantibodies against a desmosomal protein complex with a calcium-sensitive epitope are characteristic of pemphigus foliaceus patients. *J Exp Med* 1987, **165:** 1719–24.

10 Wolff K, Schreiner E Ultrastructural localization of pemphigus autoantibodies within the epidermis. *Nature* 1971; **229:** 59–61

11 Takigawa M, Imamura S, Ofuji S. Surface distribution of pemphigus antibody-binding substance(s) on isolated guinea pig epidermal cells An immunoferritin electron microscopic study. *J Invest Dermatol* 1978; **71:** 182–5.

12 Patel HP, Diaz LA, Anhalt GJ *et al.* Demonstration of pemphigus antibodies on the cell surface of murine epidermal cell monolayers and their internalization. *J Invest Dermatol* 1984; **83:** 409–15.

13 Iwatsuki, Takigawa M, Imaizumi S, Yamada M. *In vivo* binding site of pemphigus vulgaris antibodies and their fate during acantholysis. *J Am Acad Dermatol* 1989; **20:** 578–82.

14 Tsukamoto T Establishment and characterization of a cell line (KU-8) from squamous cell carcinoma of the penis. *Keio J Med* 1989; **38:** 277–93.

15 Breton-Gorius J, Vanhaeke D, Pryzwansky KB *et al.* Simultaneous detection of membrane markers with monoclonal antibodies and peroxidatic activities in leukaemia: ultrastructural analysis using a new method of fixation preserving the platelet peroxidase. *Br J Haematol* 1984; **58:** 447–58.

16 Hashimoto T. Experimental suprabasal bulla formation in organ cultured human skin with low calcium medium. *J Invest Dermatol* 1988; **90:** 501–4.

17 Jones JCR, Arnn J, Staehelin LA, Goldman RD. Human autoantibodies against desmosomes: possible causative factors in pemphigus. *Proc Natl Acad Sci USA* 1984; **81:** 2781–5.

18 Jones JCR, Yokoo KM, Goldman RD. A cell surface desmosome-associated component: identification of a tissue-specific cell adhesion molecule. *Proc Natl Acad Sci USA* 1986; **83:** 7282–6.

19 Gorbsky G, Cohen S, Steinberg MS. Desmosomal antigens are not recognized by the majority of pemphigus autoimmune sera. *J Invest Dermatol* 1983, **80:** 475–80.

20 Koch PJ, Walsh MJ, Schmelz M *et al.* Identification of desmoglein, a constitutive desmosomal glycoprotein, as a member of the cadherin family of cell adhesion molecules. *Eur J Cell Biol* 1990; **53:** 1–12.

21 Citovsky V, Naishtat S, Timberg R *et al.* The cellular response of human and guinea-pig keratinocytes to pemphigus IgG-cell death related to IgG-receptor surface migration and internalization *J Invest Dermatol* 1983; **80:** 366.

22 Kitajima Y, Inoue S, Yaoita H. Effects of pemphigus antibody on the regeneration of cell-cell contact in keratinocyte cultures grown in low to normal Ca^{2+} concentration. *J Invest Dermatol* 1987; **89:** 167–71.

23 Konohana I, Konohana A, Xia P *et al.* Expression of pemphigus vulgaris antigen in cultured human keratinocytes: effect of inhibitors, tunicamycin, and lectins. *J Invest Dermatol* 1988; **90:** 708–15.

24 Hoffman HT, Subnani M, Cha M *et al.* Calcium regulation of antigen expression on normal and malignant human squamous cells *in vitro*. *Arch Otolaryngol Head Neck Surg* 1990; **116:** 299–303.

25 Matis WL, Anhalt GJ, Diaz LA *et al* Calcium enhances the sensitivity of immunofluorescence for pemphigus antibodies. *J Invest Dermatol* 1987; **89:** 302–4.

26 Ogawa H, Taneda A, Morioka S. Characterization of pemphigus antigen. *J Invest Dermatol* 1978; **70:** 194–6.

British Journal of Dermatology (1991) 125, 238–242.
ADONIS 000709639100208A

Loss of leucocyte common antigen (CD45) on atypical lymphocytes in the localized but not disseminated type of Pagetoid reticulosis

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Accepted for publication 11 April 1991

Summary

Atypical intraepidermal T cells in Pagetoid reticulosis did not express CD45RO, the low molecular isoform of CD45. These cells did express other CD45 isoforms in the disseminated aggressive variant (Ketron–Goodman type). The complete CD45 family was lost from the cell surface in the benign localized variant (Woringer–Kolopp type). Because the presence of CD45 is crucial for the function of the lymphocyte-specific tyrosine kinase p56^{lck}, which is associated with lymphocyte growth and transformation, this may be the molecular basis for the non-aggressive nature of this clonal T-cell proliferation.

Pagetoid reticulosis is a rare, but well-defined lymphoproliferative disorder involving the skin.[1] Within the epidermis there are medium- to large-sized highly activated T cells which may be CD4$^+$, CD8$^+$ or CD4/CD8 double-negative. There is also a reactive mononuclear infiltrate beneath the epidermis.[1,2] There are clinically two variants: the localized Woringer–Kolopp type, in which there are chronic and indolent plaques that are localized predominantly on the forearms and lower legs,[3] and a more aggressive and disseminated form, the Ketron–Goodman type, that may eventually involve the lymph nodes and other organs.[4] These two variants cannot be distinguished by their histology or immunophenotype, but the presence of a solitary lesion is suggestive for the benign Woringer–Kolopp type. Recent gene-rearrangement studies of such a localized case has demonstrated that the T cells in Pagetoid reticulosis represent a clonal population[5] and the condition can be grouped with other clonal T-cell proliferative diseases of the skin with an indolent course, such as lymphomatoid papulosis.

In a recent study[6] we found that two cases with localized and two with disseminated Pagetoid reticulosis lacked the expression of CD45RO, the marker molecule for memory T cells.[7] This is unusual, because the invading T cells are usually predominantly of the memory phenotype.[8,9]

We have demonstrated the lack of expression of CD45RO on atypical cells in the localized type of Pagetoid reticulosis. This is not due to aberrant expression of other CD45 isoforms such as CD45RA or CD45RB, but based on the loss of the complete CD45 complex from the cell surface. CD45 functions as an enzyme that dephosphorylates receptors like CD2, CD3, CD4, CD8 and kinases associated with these molecules.[10,11] As a result of CD45 action, most molecules are functionally downregulated. Within the CD45 negative murine T-lymphoma cells protein kinase p56^{lck}, associated with lymphocyte transformation, is inactive.[12] We suggest that the loss of CD45 expression on the cells in the localized type of Pagetoid reticulosis could be the molecular basis for its rather indolent clinical course.

Methods

Specimens

Skin biopsies were taken from the involved skin of four patients with Pagetoid reticulosis under local anaesthesia and prior to systemic or topical treatment. Informed consent was obtained from the patients. The biopsies were immediately divided after excision and one-half fixed in 5% formaldehyde and routinely processed and embedded in paraffin, while the other half was snap frozen in liquid nitrogen and then stored at −80°C. Serial 6-μm sections were cut from each block

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Table 1. Clinical data of four patients with Pagetoid reticulosis

Case	Type	Age	Sex	Localization	Duration
1	KG	82	F	Disseminated	6 years
2	KG	65	M	Disseminated	1 year
3	WK	31	M	Right hand	5 years
4	WK	26	M	Left forearm	3 months

KG, Ketron–Goodman type; WK, Woringer–Kolopp type.

on a cryostat and were then air-dried for 24 h and stored at −20°C until used. Sections were also cut from the paraffin-embedded material and stained with haematoxylin and eosin, Giemsa and PAS.

The clinical details of the four patients are shown in Table 1 (three of the cases have been reported in detail elsewhere.[1])

In addition, biopsies were taken from two patients with mycosis fungoides where there was a variable proportion of intraepidermal atypical lymphocytes that showed a lack of CD45RO expression[6] and were used for further investigation of CD45 isoform expression.

Indirect immunoperoxidase procedure

Cryostat sections. These were fixed in acetone for 10 min at room temperature and then air-dried. Demonstration of binding of the primary monoclonal antibody was performed as previously described.[13]

Paraffin-embedded specimens. These were stained with the same indirect three-step immunoperoxidase technique.

Monoclonal antibodies

The monoclonal antibodies used are listed in Table 2. Horse-radish peroxidase-conjugated rabbit anti-mouse Ig antibodies and goat anti-rabbit Ig antibodies were purchased from Dakopatts (Denmark) and Medac (Hamburg), respectively.

Results

Histological features

All four cases of Pagetoid reticulosis showed on histology a highly epidermotropic lymphohistiocytic infiltrate with a band-like involvement of the papillary dermis (Fig. 1a) and the lower dermis was almost free of infiltrating cells. The intraepidermal lymphocytes were medium to large in size with abundant pale cytoplasm and with an irregularly shaped nucleus (Fig. 1a). The atypical lymphocytes in the epidermis occurred as single cells and in nests producing the characteristic Pagetoid appearance.

Phenotypical characterization

The dermal infiltrate in all the four cases showed a similar phenotype and consisted of moderately activated helper T cells of the memory phenotype, CD3$^+$, CD4$^+$, CD45RO$^+$, together with an admixture of macrophages. However, in contrast the phenotype of the intraepidermal infiltrate varied in the cases. Two patients (Cases 2 and 3) had CD4/CD8 double-negative T cells, while Case 1 had CD4$^+$ CD8$^-$ and case 4 had CD4$^-$ CD8$^+$ cells (Table 3).

Table 2. Monoclonal antibodies as used in this study

Antigen designation	MAb	Antigen distribution	P	Source
CD3	Leu 4	All T cells	–	BD
CD4	Leu 3	Helper T cells, activated macrophages	–	BD
CD8	Leu 2	Suppressor T cells	–	BD
CD29	4B4	β chain of VLA-integrins	–	Coulter
CD43	MT1	All T cells; macrophages	+	Dako
CD45	αLCA	All bone marrow derived cells; not erythrocytes	+	Dako
CD45RO	UCHL1	Memory T cells; macrophages	+	Dako
CD45RA	Leu 18	Naive T cells; B cells; few macrophages	–	BD
CD45RB	4KB5	B cells; T-cell subset	+	Dako
CD45R-like	Ki-B3	B cells; T-cell subset, subset of macrophages	+	A.C.Feller

P, antigen can be demonstrated in paraffin-embedded tissue. BD, Becton Dickinson, Heidelberg, Germany; Dako, Dakopatts, Hamburg, Germany. Dr A.C.Feller, Department of Pathology, University of Würzburg, Germany, kindly provided Ki-B3 MAb.

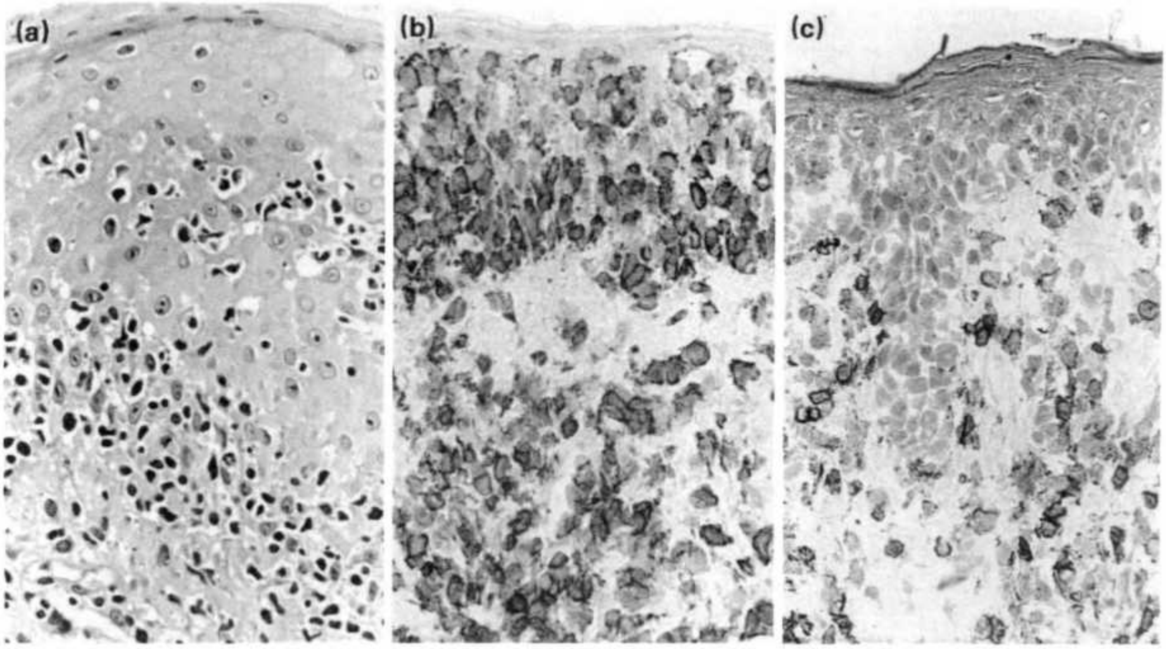

Figure 1. (Case 2) Disseminated Pagetoid reticulosis. (a) Numerous pleomorphic cells infiltrating the epidermis (haematoxylin and eosin, × 400). (b) CD45 expression on all intraepidermal T cells (immunoperoxidase on cryostat section, × 400). (c) Lack of CD45RO expression on intraepidermal T cells (immunoperoxidase on cryostat section, × 400).

Table 3. Phenotypical details of investigated cases

Diagnosis	CD45	CD45RO	CD45RA	CD45RB	CD45R-like	CD43	CD29	CD4	CD8
PR 1 (KG)	ND	–	ND	–	–	+	+	+	–
PR 2 (KG)	+	–	–	–	–	–	+	–	–
PR 3 (WK)	–	–	–	–	–	+	+	–	–
PR 4 (WK)	–	–	–	–	–	+	+	–	+
MF 1	+	–	–	–	–	+	+	+	–
MF 2	+	–	–	–	–	+	+	+	–

+, more than 60% of atypical intraepidermal cells are Ag-positive; –, less than 40% of atypical intraepidermal cells are Ag-positive; ND, not done; PR, Pagetoid reticulosis; KG, Ketron–Goodman type; WK, Woringer–Kolopp type; MF, mycosis fungoides.

All the cases of Pagetoid reticulosis and the two selected cases with mycosis fungoides showed a lack of expression of CD45RO on the intraepidermal T cells. Although in Case 1, with disseminated Pagetoid reticulosis, there was no more material for further staining, Case 2 was clearly positive for CD45 (Fig. 1b) despite the lack of expression of CD45RO (Fig. 1c). Similarly as in disseminated Pagetoid reticulosis, the intraepidermal cerebriform T cells in the two cases of mycosis fungoides that lacked CD45RO expression were positive for CD45 (pan leucocyte antigen). These cells failed to express CD45RA and CD45RB, as would be expected for peripheral memory CD4$^+$ T cells (Table 3). Staining with antibodies against different isoforms of CD45, including common and restricted antigens, revealed that the atypical intraepidermal T cells in Case 3 (Fig. 2b and c) and Case 4 gave completely negative reactions (Table 3). CD43 (MT1) was demonstrated on all atypical intraepidermal T cells in three of the four cases of Pagetoid reticulosis (Fig. 2a), and in one of the cases with mycosis fungoides, irrespective of whether there was loss of expression of CD45 (Table 3).

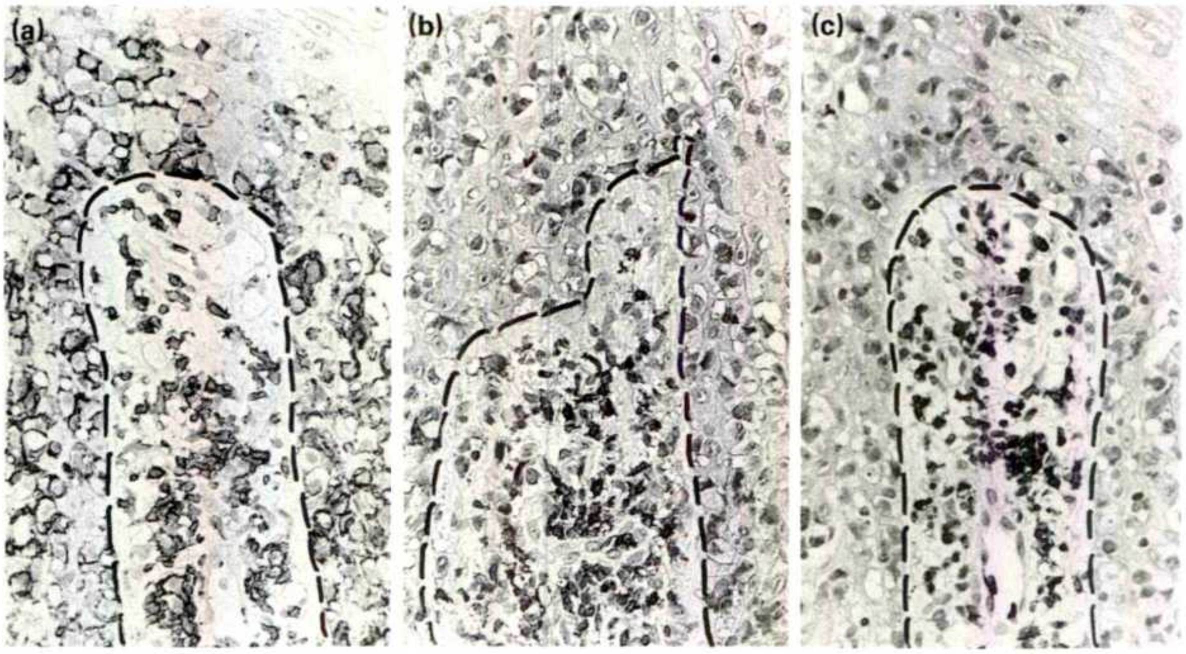

Figure 2. (Case 3) Localized Pagetoid reticulosis. (a) Demonstration of CD43 (MT1) on atypical intraepidermal and small dermal T cells. The interrupted line indicates the dermo-epidermal junction (immunoperoxidase on paraffin section, × 400). (b) Lack of CD45 expression on atypical intraepidermal T cells (immunoperoxidase on paraffin section, × 400). (c) Lack of CD45RO expression on atypical intraepidermal T cells (immunoperoxidase on paraffin section, × 400).

Discussion

In this study we have demonstrated that the atypical intraepidermal T cells in localized Pagetoid reticulosis are characterized by the complete loss of expression of CD45 molecule complex. This finding is unique in skin lymphomas, and could be used to distinguish the localized type of Pagetoid reticulosis from other cutaneous T-cell lymphomas.[14] Also in extracutaneous T-cell lymphomas, the complete loss of expression of CD45 is rare and has so far only been described in one case of adult T-cell lymphoma/leukaemia and in about 40% of large-cell anaplastic lymphomas.[15]

The differential expression of the various CD45 isoforms is precisely regulated during T-cell development,[16] and those in the thymus and at the periphery that have not yet encountered their respective antigen (naive T cells) predominantly express the restricted high molecular CD45RA isoform. After the first activation event, most likely induced by presentation of the specific antigen, naive T cells undergo profound phenotypical and functional changes that result in the expression of numerous adhesion molecules with downregulation of the CD45RA isoform and the increased expression of the 180-kDa CD45RO isoform.[7] The process is irreversible, and the T cell is then left in the memory state and the CD45RO is the only isoform that remains markedly expressed.

The lack of expression of both CD45RA and CD45RO in Pagetoid reticulosis is unique, because T cells expressing neither isoform of the CD45 molecule family have not so far been observed in the thymus[17] or peripherally.[18] Even more surprising is that the intraepidermal T cells in the localized Pagetoid type of reticulosis failed to express any CD45 molecules, because in normal T cells about 10% of the membrane proteins belong to this family.[19] As atypical lymphocytes in all cases showed strong CD29 expression, they probably belong to the memory maturation stage and may have lost CD45RO from their cell surface during the evolution of the disease. However, our cases of disseminated Pagetoid reticulosis as well as the two that were included with mycosis fungoides clearly demonstrate that the lack of CD45RO in memory T cells was not invariably associated with the complete loss of CD45 expression.

CD45 family members possess a large cytoplasmic domain that is made up of two functionally similar subunits that act as protein tyrosine phosphatases (phosphotyrosine phosphatases).[10,11] Cross-linking of CD45 with other T-cell surface molecules results in most cases in downregulation.[20] For example, loss of expression of CD45 renders T cells insensitive *in vitro* to T-cell-receptor-mediated stimulation.[21] Among the various molecules dephosphorylated by CD45 is the scr-related T-cell specific tyrosine kinase $p56^{lck}$, which plays a crucial role in T-cell growth and transformation.[22] Phosphorylation of the carboxy-terminal located Tyr-505 residue suppresses both tyrosine protein kinase activity and the oncogenic potential of $p56^{lck}$, while dephosphorylation plays an important role in triggering the $p56^{lck}$-mediated transformation.[12] Recent studies on mouse CD45$^+$ lymphoma lines and their corresponding CD45$^-$ mutants indicate that the loss of CD45 expression in the mutant lymphoma cells correlates with the increased phosphorylation of tyrosine protein kinase $p56^{lck}$ at the Tyr-505 residue, the putative negative regulatory site.[12] Therefore, despite the fact that intraepidermal T cells in Pagetoid reticulosis express numerous activation markers and many express the proliferation-associated nuclear antigen Ki67 (data not shown), the loss of CD45 expression could be the molecular basis to explain the relatively long and benign clinical course.

Acknowledgments

We thank Mrs S.Heimrich for excellent technical assistance. This work was supported by the Schleswig-Holsteinische Krebsgesellschaft.

References

1 Mielke V, Wolff HH, Winzer M, Sterry W. Localized and disseminated pagetoid reticulosis. Diagnostic immunophenotypical findings. *Arch Dermatol* 1989; 125: 402–6.
2 Braun-Falco O, Marghescu S, Wolff HH. Pagetoide Retikulose. Morbus Woringer–Kolopp. *Hautarzt* 1973; 24: 11–21.
3 Woringer F, Kolopp P. Lésion érythmato-squameuse polycyclique de l'avant-bras voluant depuis 6 ans chez un garconnet de 13 ans. Histologiquement infiltrat intra-épidermique d'apparance tumorale. *Ann Dermatol Syph* 1939; 10: 945–58.
4 Ketron LW, Goodman MH. Multiple lesions of the skin apparently of epithelial origin resembling clinically mycosis fungoides. *Arch Dermatol Syph* 1931; 24: 758–85.
5 Wood GS, Weiss LM, Hu CH *et al.* T-cell antigen deficiencies and clonal rearrangements of T-cell receptor genes in pagetoid reticulosis (Woringer–Kolopp disease). *N Engl J Med* 1988, 318: 164–7.
6 Hauschild A, Sterry W. Formalin resistant leukocyte surface antigens in the diagnosis of cutaneous malignant lymphomas *Am J Pathol* 1989; 135: 177–84.
7 Sanders ME, Makgoba MW, Shaw S. Human naive and memory T cells: Reinterpretation of helper–inducer and suppressor–inducer subsets. *Immunol Today* 1988; 9: 195–9
8 Sterry W, Mielke V. CD4$^+$ cutaneous T cell lymphomas show the phenotype of helper/inducer T cells (CD45R$^-$, CDw29$^+$). *J Invest Dermatol* 1989; 93: 413–6.
9 Brasch J, Mielke V, Sterry W. Preferential immigration of CD4$^+$ memory T cells in early positive patch test reactions. *Arch Dermatol Res* 1990; 281: 550.
10 Clark EA, Ledbetter JA. Leukocyte cell surface enzymology: CD45 (LCA, T200) is a protein tyrosine phosphatase *Immunol Today* 1989; 10: 225–8.
11 Charbonneau H, Tonks NK, Walsh KA, Fischer EH. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. *Proc Natl Acad Sci USA* 1988, 85: 7182–6.
12 Ostergaard HL, Shackelford DA, Hurley TR *et al* Expression of CD45 alters phosphorylation of the lck-encoded tyrosine protein kinase in murine lymphoma T-cell lines *Proc Natl Acad Sci USA* 1989; 86: 8959–63.
13 Stein H, Bonk A, Tolksdorf G *et al.* Immunohistologic analysis of the organization of normal lymphoid tissue and non-Hodgkin's lymphomas. *J Histochem Cytochem* 1980; 28: 746–60.
14 Sterry W. Diagnostik der kutanen malignen Lymphome. *Dtsch med Wschr* 1988; 113: 184–9.
15 Feller AC Phenotypic analysis of human T-cell lymphoma with respect to cell differentiation and morphological classification. Stuttgart, New York: Fischer, 1989.
16 Thomas ML, Lefrancois L. Differential expression of the leukocyte-common antigen family. *Immunol Today* 1988; 9: 320–6.
17 Pilarski LM, Deans JP. Selective expression of CD45 isoforms and of maturation antigens during human thymocyte differentiation: observations and hypothesis. *Immunol Letters* 1989; 21: 187–98.
18 Pulido R, Cebrian M, Acevado A *et al.* Comparative biochemical and tissue distribution study of four distinct CD45 antigen specificities. *J Immunol* 1988; 140: 3851–7.
19 Hall LR, Streuli M, Schlossman SF, Saito H. Complete exon-intron organization of the human leukocyte common antigen (CD45) gene *J Immunol* 1988; 141: 2781–7.
20 Ledbetter JA, Tonks NK, Fischer ED, Clark EA. CD45 regulates signal transduction and lymphocyte activation by specific association with receptor molecules on T and B cells. *Proc Natl Acad Sci USA* 1988; 85: 8628–32.
21 Koretzky GA, Picus J, Thomas ML, Weiss A. Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway. *Nature* 1990; 346: 66–8.
22 Alexander DR, Cantrell DA Kinases and phosphatases in T-cell activation. *Immunol Today* 1989; 10: 200–5.

British Journal of Dermatology (1991) 125, 243–247.

ADONIS 000709639100209N

The influence of cutaneous factors on the transcutaneous pO_2 and pCO_2 at various body sites

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Accepted for publication 27 March 1991

Summary

The transcutaneous partial pressures of oxygen ($tcpO_2$) and carbon dioxide ($tcpCO_2$) were measured at eight different sites in 10 adult male subjects with an electrode at a temperature of 44°C. The mean $tcpO_2$ values (mmHg) were significantly lower on the face (forehead 26·6, cheek 29·6) and the palm (26·4) than at other sites (60·6–69·6). The $tcpCO_2$ values (mmHg) were only slightly higher on the face. Removal of the stratum corneum produced an average increase of the $tcpO_2$ on the palm of 37·6 mmHg and on the forehead of 19·6 mmHg. However, in 10 children with an age range of 3–9 years, the difference in the mean pO_2 between the cheek and forearm was very small. There was no significant difference in the cutaneous blood flow at 44°C between the cheek, palm and forearm.

The transcutaneous partial pressure of oxygen ($tcpO_2$) and that of carbon dioxide ($tcpCO_2$) can be readily measured by non-invasive techniques that are now widely used in the management of premature infants. The $tcpO_2$ and $tcpCO_2$ values can be influenced by various factors,[1] including the condition of the skin at which the probe is applied. This study was performed to confirm and extend previous studies[2,3] as to the normal values and the variations of $tcpO_2$ at various body sites, and to investigate the reason for the low $tcpO_2$ levels as recorded on the face and palms.

Methods

Determination of $tcpO_2$ and $tcpCO_2$ values in adults

Ten healthy male volunteers (age range 24–36 years, mean age 29 years) were studied. The $tcpO_2$ and $tcpCO_2$ values were simultaneously measured on the forehead, cheek, palm, anterior aspect of the forearm, abdomen (3 cm above the umbilicus), back (10 cm above the iliac crest), tibia and the posterior aspect of the leg. A module 632 $tcpO_2$ monitor and a module 634 $tcpCO_2$ monitor (Kontron, Zurich, Switzerland) with thermostatically controlled electrodes set at 44°C were used. The probes were fixed to the skin using double-sided adhesive tape after the skin was degreased with alcohol. Twenty minutes were allowed for stabilization before the $tcpO_2$ and $tcpCO_2$ values were determined. All measurements were carried out in a room where the temperature was regulated at 25°C and the humidity was about 50%. The subjects were forbidden to smoke for 2 h before the examination and remained supine or prone during the course of the study.

Cutaneous blood flow in the forearm, cheek and palm

The cutaneous blood flow at 44°C (CBF_{44}) was measured using the ALF 2100 laser-Doppler flowmeter (Advance, Tokyo, Japan) on the cheek, forearm and palm of these 10 subjects. The probe holder was specially constructed to be the same size as the $tcpO_2$ probe and could be thermostatically controlled to 44°C. The blood flow value was determined 20 min after the application of the probe and this apparatus could detect blood flow at a depth of about 1 mm from the skin surface.[4]

Removal of the stratum corneum and measurements of $tcpO_2$

Five subjects with low facial and palmar $tcpO_2$ values were selected from our 10 subjects and were further examined to determine the influence of the stratum corneum at these sites on the $tcpO_2$. The facial stratum corneum was removed by tape stripping until the skin surface became uniformly shiny. Immediately and approximately 24 h after tape stripping, the $tcpO_2$ measurements were made. After the first measurements, the test sites were occluded with plastic film to prevent crust formation before the second $tcpO_2$ determination.

As the stratum corneum was not readily removed

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from the hypothenar eminence on the palm by tape stripping, we used 50% salicylic acid plasters cut to the same size as the tcpO_2 probe, applied for 48 h. The stratum corneum was then easily removed and this was confirmed on histology of a punch biopsy taken at the edge of a treated site and which showed the extent of the removal of the stratum corneum.

Soon after the measurement of the tcpO_2 at this treated site, the response of the tcpO_2 after arterial occlusion using a sphygmomanometer cuff set at 200 mmHg was also recorded. The reduction of the tcpO_2 after arterial occlusion was determined to estimate local oxygen consumption of the skin.[5]

Cheek and forearm tcpO_2 values in children

The tcpO_2 on the cheek and forearm of 10 healthy children (four boys and six girls; age range 3–9 years, mean age 5 years) was also measured.

Statistical analysis

In all experiments the data were analysed using the paired *t*-test, and a $P<0{\cdot}01$ was considered significant.

Results

tcpO_2 and tcpCO_2 in adults

The mean tcpO_2 values determined at each location are shown in Table 1. The mean values determined at three sites (forehead, cheek and palm) were significantly lower than those for the other five sites. These other sites showed no significant differences between any pair of them, except for forearm versus abdomen ($P=0{\cdot}003$).

The mean tcpCO_2 values are also shown in Table 1. The values for the forehead and cheek were slightly but significantly higher than those determined at other sites.

Table 1. tcpO_2 and tcpCO_2 at eight body sites (mean ± SD)

Site	tcpO_2 (mmHg)	tcpCO_2 (mmHg)
Forehead	26·6±21·0	67·2±2·4
Cheek	29·6±9·8	69·2±2·8
Volar aspect of the forearm	69·6±5·3	62·6±3·3
Abdomen	63·8±6·4	63·7±2·4
Back	60·6±12·2	63·9±3·1
Subcutaneous border of the tibia	66·6±6·1	61·1±4·1
Posterior aspect of the leg	67·3±6·8	62·8±2·9
Palm	26·4±6·6	60·5±3·1

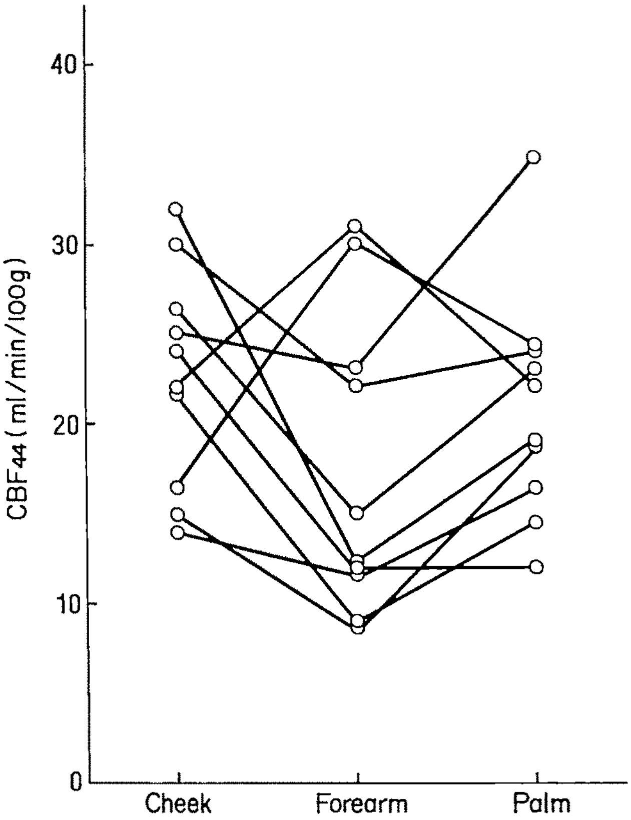

Figure 1. Cutaneous blood flow at 44°C determined with laser-Doppler flowmeter on cheek, forearm and palm.

The other six sites showed no significant differences between any pair of them, except for back versus palm ($P=0{\cdot}009$).

Cutaneous blood flow in the forearm, cheek and palm

The mean CBF_{44} values (mean ± SD, ml/min/100 g) for the cheek, forearm and palm were $22{\cdot}7\pm6{\cdot}1$, $17{\cdot}5\pm8{\cdot}4$ and $20{\cdot}9\pm6{\cdot}4$, respectively. No significant differences were noted between any pair of these values ($P\geqslant0{\cdot}1$, Fig. 1), although this may be due to two subjects whose CBF_{44} in the forearm were rather high.

Effect of removal of stratum corneum on tcpO_2

On the forehead, the mean value of tcpO_2 was reduced immediately after stripping (6·6 mmHg), whereas it increased significantly (33·2 mmHg) after 24 h when compared with the value before stripping (13·6 mmHg) (Table 2). The tcpO_2 recorded immediately after stripping was regarded as inadequate for the accurate evaluation of the effect of stripping because the test site appeared

Table 2. The change in tcpO$_2$ after stripping the stratum corneum on the forehead (mean ± SD, $n=5$) and tcpO$_2$ and its rate of reduction during arterial occlusion before and after the removal of the stratum corneum on the palm (mean ± SD, $n=5$)

Change in tcpO$_2$ (mmHg)	Before	Immediately after	After 24 h
Forehead	13·6 ± 13·3	6·6 ± 5·4	33·2 ± 9·5
Palm	30·6 ± 9·2	68·2 ± 10·3	
Reduction rate (mmHg/min)	5·4 ± 3·4	51·4 ± 12·8	

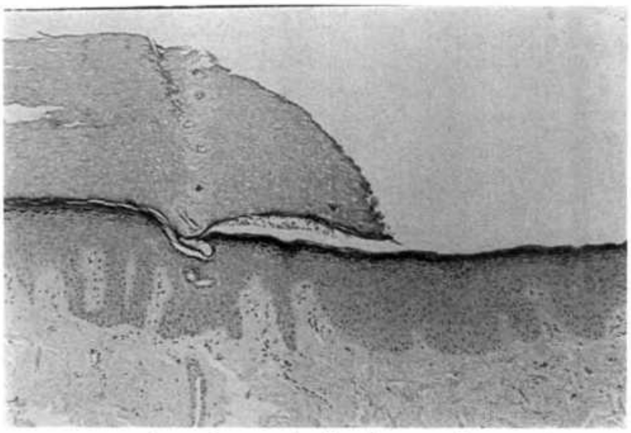

Figure 2. Histology of stripped palm showing absence of stratum corneum at the level of the stratum lucidum (right) with no inflammatory changes.

oedematous. However, even after 24 h the tcpO$_2$ of the forehead was still significantly lower than that of the forearm without stripping (Table 2).

On histology, the stratum corneum was found to be removed at the level of the stratum lucidum in the palm (Fig. 2). No inflammatory changes were found in the upper dermis. The mean tcpO$_2$ value for the palm where the stratum corneum had been removed was about 40 mmHg higher than the value obtained before the removal. The rate of reduction of tcpO$_2$ during arterial occlusion was also markedly influenced by the removal of the stratum corneum, which caused an average increase of about 45 mmHg/min (Table 2).

tcpO$_2$ of the cheek and forearm in children

The tcpO$_2$ values for the cheek and forearm in adults and children are compared in Figure 3. In the children, the mean tcpO$_2$ was slightly higher in the forearm (76·3 ± 5·3 mmHg) than in the cheek (71·6 ± 7·9 mmHg) ($P=0·03$). However, the difference was very small when compared with that seen in the adults (cheek 29·6 ± 9·8 mmHg; forearm 69·6 ± 5·3 mmHg).

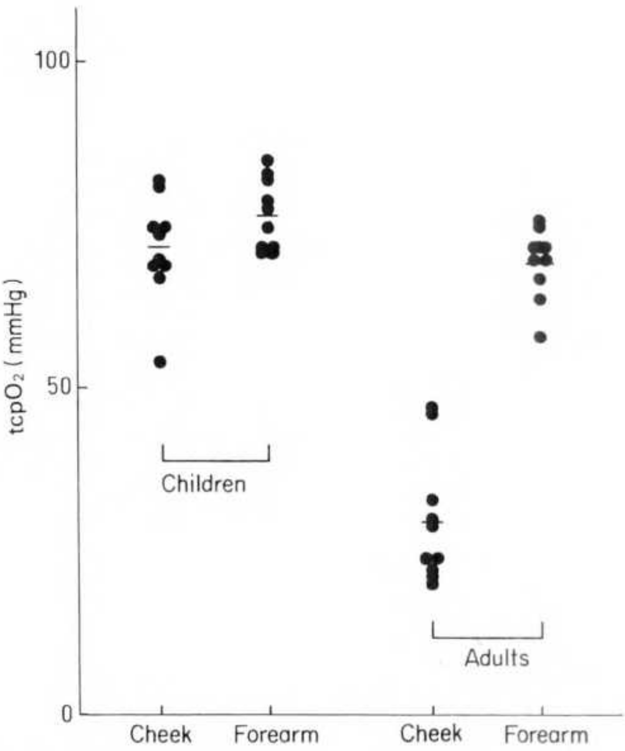

Figure 3. The tcpO$_2$ values recorded on the cheek and forearm of healthy children and adults.

Discussion

The tcpO$_2$ and tcpCO$_2$ have been measured in a number of skin conditions such as leg ulcers,[6,7] morphoea and scleroderma,[8,9] hypertrophic scars[8,10] and necrobiosis lipoidica.[11] The values represent the partial pressures of oxygen and carbon dioxide at the level of the skin, and these can be influenced by many factors, such as the blood flow, conductivity of the gases and the production and consumption of them in the skin. Heating the skin to a temperature of 44°C is used to achieve 'arterialization' in the capillaries, inducing vasodilatation and abolishing the normal reflex vasoconstriction.[12] The temperature setting of the electrode at 37°C has been recommended for the investigation of the tcpO$_2$ dynamics in patients with venous leg ulcers,[12] but in this present study, both tcpO$_2$ and tcpCO$_2$ were measured at 44°C.

Our results are similar to those of previous reports,[2,3] and there was little difference in the tcpO$_2$ for sites on the trunk, legs and arms. However, the tcpO$_2$ on the forehead, cheek and palm were much lower than those at other body sites. Because blood pO$_2$ does not vary in the arteries, the low tcpO_2 on the face and palm appears to be due to local factors. Falstie-Jensen *et al.*[3] reported that the thickness of the living part of the epidermis correlated with the tcpO_2, and the change in oxygen tension across it was 0·26 mmHg/μm. The relationship between the tcpO_2 and the total epidermis, including the stratum corneum, was not examined in this report. Our results could be explained if the living part of the epidermis on the face and palm was three times more thick than at other sites.[3] However, the thickness of the epidermis on the face is the same as in other locations in Japanese subjects,[13] and the palmar epidermis not only has a thicker living layer but also a thick stratum corneum. Because the tcpO_2 is mainly influenced by the blood flow of superficial capillaries,[14] the flow was measured in the cheek, palm and forearm at 44°C but we found no differences between the three sites. After removal of the stratum corneum on the palm, the tcpO_2 was now similar to that found at other body sites. Thus, the thick stratum corneum appeared to play an important role in reducing the palmar tcpO_2.

Why should the stratum corneum, which consumes no oxygen, cause a reduction of the tcpO_2 value? If the tcpO_2 electrode (Clark-type platinum electrode) itself consumed no oxygen, then theoretically there would be no difference between the tcpO_2 (i.e. the supracorneal pO_2) and the subcorneal pO_2, because the stratum corneum only delays the diffusion of oxygen. However, the reaction in the electrode ($O_2 + 2H_2O + 4e^- = 4OH^-$) consumes oxygen and produces an oxygen diffusion field[15] and a pO_2 gradient in the stratum corneum (diffusion error[16]). Jaszczak and Sejrsen[17] reported that stripping of the skin increased the mean tcpO_2 (45°C) value by 27 mmHg even on the forearm, where the stratum corneum is much thinner than on the palm. We also observed an average increase of 38 mmHg when the stratum corneum was removed from the palm to the level of the stratum lucidum. Therefore, it is possible that the thicker the stratum corneum, the more marked is the difference in tcpO_2 between the outer and inner parts of this layer. Moreover, as the tcpO_2 fell faster during arterial occlusion when most of the stratum corneum was removed, the rate of fall does not reveal the true oxygen consumption rate in the skin when there is a thick stratum corneum. Our results suggest that the thickness and other characteristics of the stratum corneum must be considered when tcpO_2 and its dynamic response are measured. It is preferable, therefore, to remove the stratum corneum by stripping or other methods before attaching the monitor when the 'real' tcpO_2 and its real-time dynamics are required, especially with hyperkeratotic skin conditions. The tcpO_2 and its dynamics could also be considered as one of the parameters of the hyperkeratotic condition.

The cause of the decreased tcpO_2 on the face is still uncertain. Orenstein *et al.*[2] also found a low tcpO_2 on the face and concluded that this might be due to partial obstruction of capillaries caused by the pressure of the electrode (10 g). However, a low tcpO_2 was also present on the cheek where no rigid skeleton lies below the skin to allow compression of the tissues, and the tcpO_2 was not low over the subcutaneous border of the tibia where the bone lies immediately beneath the skin.

The main histological difference between the skin of the face and the forearm is the existence of many well-developed sebaceous glands. The density of sebaceous glands in the face is 5–10 times higher than in the forearm in Japanese subjects.[18] The oxygen measured by the present method is carried via the blood and then diffuses from the capillaries to the skin surface. If the sebaceous glands consume a large amount of oxygen during the diffusion process, the tcpO_2 may well be reduced. To test this hypothesis, we measured tcpO_2 on the cheek in children, because the sebaceous glands are not well developed[19] and sebum production is decreased[20] during childhood. Little reduction of the tcpO_2 was observed on the cheek in children, indicating that the reduction noted in adults could be due to the high consumption of oxygen by sebaceous glands. However other possibilities, such as epidermal factors and the existence of numerous anastomoses in the cheek,[21] could also cause a reduction of facial tcpO_2 in adults. The facial epidermis is not thick but the oil content of the stratum corneum may differ between the face and other sites, and between adult and child. An increase in tcpO_2 was observed on the face after stripping of the stratum corneum, but the value was still significantly lower than that on the forearm. This indicates that the stratum corneum is of less importance in the reduction of tcpO_2 on the face. We have no data about the ratio of the blood flow through anastomoses in the measured site heated with the probe, and whether arteriovenous shunting would affect the facial and palmar tcpO_2. The effect of arteriovenous shunting on tcpO_2 needs to be examined further.

There were no marked differences between the various sites tested as to the tcpCO_2, but a slight but statistically significant increase was found on the forehead and cheek. This may also be related to the metabolism of sebaceous glands in the adult, with an increase in the production of CO_2. The possible reasons why tcpCO_2 showed less changes than tcpO_2 are as follows: (i) CO_2 is 20-fold more soluble than O_2 in water[22] and so is more rapidly transported into the capillaries and removed by the blood. (ii) The pCO_2 can be kept stable by the buffering effect of the blood. (iii) No diffusion error is induced by the $tcpCO_2$ electrode (a pH-sensitive glass electrode), so the $tcpCO_2$ is not influenced by a thick stratum corneum.

Cutaneous blood flow and local factors such as the thickness of the epidermis including the stratum corneum and the metabolism of the epidermis and glands, all appear to affect the transcutaneous gas pressure, especially $tcpO_2$. These factors should therefore be considered when $tcpO_2$ and $tcpCO_2$ data are obtained from lesions at skin sites with a thick epidermis or a large number of skin appendages, especially when they must be used as a substitute for the arterial gas pressure. However, further investigation of the influence of various pathophysiological changes on $tcpO_2$ and $tcpCO_2$ should allow these parameters to be used as reliable markers of the condition of the skin.

References

1 Lubbers DW, Grossmann U. Gas exchange through the human epidermis as a basis of $tcPO_2$ and $tcPCO_2$ measurements. In: *Continuous Transcutaneous Blood Gas Monitoring* (Huch R, Huch A, eds). New York: Marcel Dekker, 1983: 1–34.

2 Orenstein A, Mazkereth R, Tsur H. Mapping of the human body skin with the transcutaneous oxygen pressure method. *Ann Plast Surg* 1988; **20**: 419–25.

3 Falstie-Jensen N, Spaun E, Brochner-Mortensen J, Falstie-Jensen S. The influence of epidermal thickness on transcutaneous oxygen pressure measurements in normal persons. *Scand J Clin Lab Invest* 1988; **48**: 519–23.

4 Kashima S, Hashizume T, Mitsui K. Properties of laser blood flowmeter. *J Jap Soc Laser Med* 1988; **9**: 3–7 (in Japanese).

5 Severinghaus JW, Stafford M, Thunstrom AM. Estimation of skin metabolism and blood flow with $tcPO_2$ and $tcPCO_2$ electrodes by cuff occlusion of the circulation. *Acta Anaesth Scand* 1978; **68** (Suppl.): 9–15.

6 Franzeck UK, Bollinger A, Huch R, Huch A. Transcutaneous oxygen tension and capillary morphologic characteristics and density in patients with chronic venous incompetence. *Circulation* 1984; **70**: 806–11.

7 Nemeth AJ, Eaglstein WH, Falanga V Clinical parameters and transcutaneous oxygen measurements for the prognosis of venous ulcer. *J Am Acad Dermatol* 1989; **20**: 186–90.

8 Takiwaki H. Transcutaneous PO_2 measurements on clinically sclerotic lesions in scleroderma and hypertrophic scar. *Nishinihon J Dermatol* 1987; **49**: 492–8 (in Japanese).

9 Silverstein JL, Steen VD, Medsger A, Falanga V. Cutaneous hypoxia in patients with systemic sclerosis (scleroderma). *Arch Dermatol* 1988; **124**: 1379–82.

10 Berry RB, Tan OT, Cooke PM *et al.* Transcutaneous oxygen tension as index of maturity in hypertrophic scars treated by compression. *Br J Plast Surg* 1985; **38**: 163–73.

11 Brüngger A. Transkutane sauerstoff- und kohlendioxiddruckgmessung bei necrobiosis lipoidica. *Hautarzt* 1989; **40**: 231–2.

12 Sarkany I, Dodd HJ, Gaylarde PM. Surgical correction of venous incompetence restores normal skin blood flow and abolishes skin hypoxia during exercise. *Arch Dermatol* 1989; **125**: 223–6.

13 Hashimoto K. Epidermis and skin appendages. In: *Nippon Hifuka Zensho* (Kitamura T, Miura O, Nakagawa K, Takahashi Y, eds), 1st edn., Vol. 1. Tokyo: Kanehara Shuppan, 1958; 7 (In Japanese).

14 Svedman P, Holmberg J, Jacobsson S *et al.* On the relation between transcutaneous oxygen tension and skin blood flow. *Scand J Plast Reconstr Surg* 1982; **16**: 133–40.

15 Baumgärtl H, Grunewald W, Lübbers DW. Polarographic determination of the oxygen partial pressure field by Pt microelectrodes using the O_2 field in front of Pt macroelectrode as a model. *Pflügers Arch* 1974; **347**: 49–61.

16 Lübbers DW. Cutaneous and transcutaneous PO_2 and PCO_2 and their measuring conditions. *Birth Defects Orig Artic Ser* 1979; **15**: 13–31.

17 Jaszczak P, Sejrsen P. Oxygen tension and consumption measured by a $tcPO_2$ electrode on heated skin before and after epidermal stripping. *Acta Anaesthesiol Scand* 1987; **31**: 362–9.

18 Hashimoto K. Local anatomy of the skin. In: *Gendai Hifuka-gaku Taikei* (Yamamura Y, Kukita J, Sano E, Seiji S, eds), 1st edn., Vol. 3A. Tokyo: Nakayama Shoten, 1982; 335 (In Japanese).

19 Morohashi M. Sebaceous gland. In: *Gendai Hifuka-gaku Taikei* (Yamamura Y, Kukita J, Sano E, Seiji S, eds), 1st edn., Vol. 3A. Tokyo: Nakayama Shoten, 1982; 197 (in Japanese).

20 Pochi PE, Strauss JS, Downing DT. Age-related changes in sebaceous gland activity. *J Invest Dermatol* 1979; **73**: 108–11.

21 Moretti C, Ellis RA, Mescon H. Vascular patterns in the skin of the face. *J Invest Dermatol* 1957; **33**: 103–12.

22 Comroe JH. Pulmonary gas diffusion. In: *Physiology and Respiration*, 2nd edn. Chicago: Year Book Medical Publishers, 1974; 159.

British Journal of Dermatology (1991) 125, 248–252.

ADONIS 000709639100210H

Psychological characteristics of women with androgenetic alopecia: a controlled study

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Accepted for publication 9 April 1991

Summary The psychological characteristics and the hair problems of 58 females with androgenetic alopecia were compared with a group of women with non-apparent dermatological diseases, and with a group of men with androgenetic alopecia. The women with androgenetic alopecia had higher scores for self-sufficiency and social inadequacy compared to women with non-visible dermatological complaints, and they scored higher for inadequacy, rigidity and general psychological maladjustment than the men and had lower scores for injuredness self-evaluation and self-esteem. The women with androgenetic alopecia had more psychosocial problems, which they attributed to the hair loss, than the other groups.

Few scientific studies have been published on the psychological problems related to hair loss, and these have mainly concerned men. A greater degree of neuroticism and psychoticism was found in men with androgenetic alopecia and who were participating in hair weaving therapy when compared with those unconcerned about their hair loss.[1] No evidence of general psychological and social difficulties was found in other studies of men with androgenetic alopecia,[2,3] although a number of specific problems closely related to hair loss were reported by a substantial proportion of men. In a study of 564 women, 13% were found to have loss of hair from the scalp before the menopause and 37% afterwards.[4] So far, there have been no empirical studies on the psychological problems of women with androgenetic alopecia. It is likely that in our society, which places much importance on the physical attractiveness of women, they would be expected to have more distress about their hair loss than men. In a study of 32 women with diffuse alopecia who were examined by a psychiatrist, more than 20% of them had severe marital and sexual problems and two were overtly depressed.[5] However, objective measurements were lacking in this study and there was no suitable control group. In the present study, the psychological and social problems of women with androgenetic alopecia were studied using standardized psychological techniques and compared with two control groups; women without visible dermatological disorders and men with androgenetic alopecia.

Methods

Subjects

The female subjects studied were involved in a trial of 2% minoxidil lotion in the treatment of their hair loss. They had diffuse thinning of the hair in the frontal and parietal regions of the scalp, with and without temporal recession, and were graded I and II according to the Ludwig scale.[6] Fifty-eight women (age range 20–47 years, mean age 35 years) participated in the psychological study.

A group of 48 female control subjects (age range 18–44 years, mean age 29 years) with non-chronic dermatological conditions were also recruited for the study.

A further control group was included that consisted of men with androgenetic alopecia types III or IV according to the Hamilton scale. This group had undergone psychological studies in a previous project which has been reported.[3] The protocol for this study was reviewed and approved by the Medical Ethics Committee of the University Hospital Rotterdam–Dijkzigt.

Procedure

All the female subjects with androgenetic alopecia who participated in our psychological study were at the end of the first 32-week period of treatment either with minoxi-

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dil or with placebo. The female control subjects had the same series of questionnaires as were given to those with androgenetic alopecia, apart from the hair problem list and instead they had a dermatological complaint list. The male control group completed the same series of questionnaires apart from the self-rating scale for depression, and the self-rating questionnaire for anxiety.

Psychological evaluation

Hair problem list. This measured psychological and social problems related to baldness and seeking help for the condition. It contained 20 items based on several in-depth interviews with the male and female subjects with androgenetic alopecia. The list was constructed in the form of a Likert scale with five response categories and the scores added.

Dermatological complaint list. This was an adaptation of the hair problem list and focused on general dermatological complaints. Three questions from the original list were excluded because they were related to hair.

Inventory list on associations with others. This was a self-reporting inventory of social anxiety and assertiveness. It contained two scales of social activities, the overall discomfort during social contact with others and the frequency of social contact. It had five subscales that concerned passing criticism, expressing personal opinions, paying compliments to others, initiating conversations and stating positive self-assertiveness. This inventory list is satisfactory as regards its reliability and validity.[7]

Self-esteem scale. This was used for measuring global self-esteem[8] and was developed from the Rosenberg self-esteem scale.[9] It contained nine items and was scored on a five-point scale.

Dutch personality questionnaire. This was a translation and shortened version of the California Psychological Inventory. There were 133 items that measured seven personality traits. These were inadequacy (IN) (vague physical complaints, depressed mood, non-specific anxiety and feelings of insufficiency), social inadequacy (SI) (avoidance of and distress regarding social contacts), rigidity (RG) (preference for situations where everything goes to one's plan, possession of fixed habits and principles), injuredness (IJ) (criticism and distrust of others), self-sufficiency (SS) (satisfaction with oneself and disinterest in others and their problems), dominance (DO) (self-confidence, preference for taking the initiative and the leading role), self-evaluation (SE) (positive attitude towards work, flexibility and well-adaptedness). The reliability of these scales has been confirmed.[10]

Delft questionnaire. This was a screening instrument for general and unspecified psychological problems. It contained 33 items and was used for obtaining a global psychological assessment for maladjustment. The reliability and validity of this questionnaire was considered to be satisfactory.[11]

The body-cathexis list. This was originally developed by Secord and Jourard[12] and measured the degree of satisfaction with various parts or processes of the body. It included 47 items, but in this study we used a translated and modified 40-item version of the list included in most recent studies.[13] Body characteristics were evaluated on a five-point Likert scale, ranging from strongly negative to strongly positive. Its reliability is considered to be satisfactory.[14]

Self-rating scale for depression. This was a Dutch translation[15] of the self-rating depression scale developed by Zung.[16] The scale included 20 items formulated either positively or negatively, which were constructed on the basis of the diagnostic criteria for depression.

Self-rating questionnaire. This was a Dutch translation of the Spielberger state–trait anxiety inventory.[17] It consists of two separate 20-item parts. The state–anxiety scale measures the intensity of feelings of anxiety at a particular moment in time. The trait–anxiety scale measures a person's general tendency towards perceiving a wide range of situations as being threatening. Research has confirmed the reliability and validity of this questionnaire.[18] In this study only the trait–anxiety scale was used, because our interest was focused on general problems and traits.

Data analyses

One-sided *t*-tests for independent samples were used for comparing the female and the male subjects with androgenetic alopecia. As the scores for inadequacy, social inadequacy, dominance and general psychological maladjustment were sex-dependent in the general population, these were adjusted in the analyses by calculating the difference between the actual score of the subjects and the mean score of the norm group of the same sex.[10] As the female dermatological control subjects were substantially younger, ANCOVAS (analysis of covariance) were performed when comparing the female subjects with androgenetic alopecia and the female dermatological subjects for each psychological measurement separately, in which age was included as a covariant. In addition, the answers given by the groups to the questions on the hair problem/dermatological problem list, were described by percentage scores.

Results

The mean psychological characteristics of the women with androgenetic alopecia, those of the female dermatological control group and the males with androgenetic alopecia are shown in Table 1.

The women with androgenetic alopecia were found to have significantly higher scores for self-sufficiency and the hair problem/dermatological complaint list when compared to the dermatological control group. They also had a trend towards higher scores for social inadequacy.

The women with androgenetic alopecia had significantly lower scores for self-evaluation and esteem compared to the men, and scored higher on general psychological maladjustment and the hair problem list. In addition, the female subjects with androgenetic alopecia showed trends towards higher scores on inadequacy and rigidity and lower scores for injuredness.

The percentage scores and absolute numbers obtained from the different groups on the hair problem/dermatological complaint list are shown in Table 2. More than half the women with androgenetic alopecia worried about their hair loss, and more than a third felt ashamed and less attractive. They were often reminded about their hair loss by others and felt uncomfortable in the presence of both men and women.

The percentage scores of women with androgenetic alopecia were substantially higher for each problem when compared with those of female control subjects. The scores were also higher than those of the men with androgenetic alopecia. Nearly five times more women were worried about their hair loss than the men, and these women were more uncomfortable in the presence of men and women than males with androgenetic alopecia. They went out less when compared with men with androgenetic alopecia. However, the men mentioned more often that they were reminded about their hair loss and that they felt older.

Discussion

Only a few studies are available on the social and psychological aspects of hair loss. Previous research into hair focused mainly on the interpersonal aspects and investigated the influence of an individual's physical attractiveness. It has been shown, for example, that blondes are expected to be less intelligent and redheads

Table 1. Mean psychological characteristics of female subjects with androgenetic alopecia, a female dermatological control group and a control group of men with androgenetic alopecia (standard deviations in parentheses); and *P* values of the differences

Psychological characteristics	Women with androgenetic alopecia (I)	Dermatological control group (II)	Men with androgenetic alopecia (III)	*P* values I vs. II	*P* values I vs. III
Inadequacy	14·5 (9·8)	12·8 (9·8)	8·5 (7·5)	NS	<0·10
Social inadequcy	10·6 (6·6)	8·1 (7·1)	8·9 (6·8)	<0·10	NS
Rigidity	26·3 (8·7)	22·5 (7·4)	24·6 (7·7)	NS	<0·10
Injuredness	17·0 (8·6)	16·7 (7·9)	18·6 (7·9)	NS	<0·10
Self-sufficiency	12·9 (6·5)	9·8 (4·2)	13·3 (5·0)	<0·05	NS
Dominance	14·4 (6·1)	16·0 (5·5)	17·1 (6·1)	NS	NS
Self-evaluation	26·3 (5·3)	27·8 (5·4)	29·6 (5·6)	NS	<0·001
Overall social discomfort	65·6 (17·3)	68·9 (19·2)	64·3 (17·6)	NS	NS
Overall social frequency	110·8 (20·1)	114·6 (19·8)	110·5 (16·3)	NS	NS
General psychological maladjustment (D.Q.)	17·3 (8·5)	15·7 (8·1)	11·2 (7·3)	NS	<0·001
Depression scale	38·9 (9·2)	34·9 (7·9)	—	NS	—
Trait anxiety	41·4 (10·6)	38·2 (10·8)	—	NS	—
Self-esteem	32·2 (6·6)	33·7 (5·6)	35·7 (4·3)	NS	<0·001
Body-cathexis	136·1 (22·3)	138·7 (20·9)	138·7 (20·9)	NS	NS
Hair problem/dermatological complaint list*	2·8 (0·8)	1·7 (0·6)	2·3 (0·7)	<0·001	<0·001

* Mean item score.
NS, not significant.

Table 2. Specific problems of women with androgenetic alopecia, a female dermatological control group and a male control group with androgenetic alopecia. Absolute numbers in parentheses*

Hair problem/dermatological complaint statements	Women with androgenetic alopecia (%)	Dermatological control group (%)	Men with androgenetic alopecia (%)
Tried medical therapy	76 (43)	54 (25)	26 (43)
Compares own hair with that of others	68 (39)	—	51 (85)
Tried remedies	58 (33)	35 (16)	42 (70)
Worries about hairloss/complaint	54 (31)	17 (8)	11 (19)
Annoyed by jokes about hairloss/complaint	47 (27)	11 (5)	28 (47)
Feels ashamed	42 (24)	15 (7)	8 (13)
Becomes reminded of hairloss/complaint	37 (21)	7 (3)	54 (90)
Others underestimate hair problems/complaint	37 (21)	11 (5)	39 (65)
Feels less attractive	37 (21)	9 (4)	29 (48)
Discomfort in presence of men	37 (21)	7 (3)	4 (6)
Discomfort in presence of women	35 (20)	2 (1)	12 (20)
Considered a wig	33 (19)	—	11 (18)
Talks frequently about hairloss/complaint	30 (17)	15 (7)	8 (13)
Considered hair transplant	26 (15)	—	20 (34)
Goes out less	23 (13)	4 (2)	5 (8)
Little understanding from others	19 (11)	9 (4)	20 (34)
Others make hairloss/complaint a problem	18 (10)	2 (1)	20 (34)
Feels much older	12 (7)	7 (3)	25 (41)
Less able to make contact	7 (4)	2 (1)	2 (4)
Feels excluded	7 (4)	2 (1)	1 (2)

* Number of subjects answering agree or strongly agree.

to be less serious,[19] and very hairy men to be more sexually potent.[20] In a study by Moerman,[21] 49 male and 47 female respondents were asked to give scores on a picture of either a man with a full head of hair or a man with fully developed androgenetic alopecia. The bald man was rated on average 10 years older than the man with hair. In addition, the respondents rated the bald man as being more intelligent, stable and conscientious, whereas the man with hair was rated as being more attractive and agreeable.

In this study, we evaluated a sample of 58 women with androgenetic alopecia who were selected from a population of women who had previously applied for hair treatment. Therefore, generalizations have to be restricted to the clinical population. Our results support the hypothesis that women suffering from androgenetic alopecia have more psychosocial problems than women with a dermatological complaint. They were also found to have more problems than male subjects with androgenetic alopecia. The finding that considerably more women with androgenetic alopecia felt ashamed, experienced discomfort in the presence of both men and women and went out less than the men and women in the control groups, indicated that these problems were particularly associated with their social life.

The differences between the results obtained from the female and male subjects with androgenetic alopecia can partly be explained by sex differences with regard to the reporting of symptoms of distress, because female subjects are less defensive about admitting to problems than male subjects.[22] However, this is not a complete explanation as several complaints mentioned by the male subjects with androgenetic alopecia were to an equal or greater degree (e.g. feels much older). The visibility of androgenetic alopecia may be the crucial factor, because in women it means that they feel less feminine and in men that they feel much older.

In several studies,[23,24] patients with visible dermatological complaints such as acne, psoriasis and eczema reported similar problems to those found in our female subjects with androgenetic alopecia. There were also a substantial number of patients who reported problems related to their social life.

On the basis of in-depth interviews with women with androgenetic alopecia (unpublished data) it was concluded that almost 30% of the women could not cope with the problems of hair loss and were preoccupied by the negative consequences. Additionally, many patients considered that the psychological support from their general practitioners was insufficient. In another study[25] about 50% of the patients who consulted their general practitioner with complaints of hair loss appeared to be suffering from psychological problems as well. However, most general practitioners followed the same policy for their patients with hair loss whether they had psychological problems or not.

Acknowledgment

This work was supported by the Upjohn Company, Kalamazoo, MI, U.S.A.

References

1 Gosselin C. Hair loss, personality and attitudes. *Pers Indiv Differ* 1984; 5: 365–9.
2 Passchier J, van der Donk J, Dutree-Meulenberg ROGM *et al.* Psychological characteristics of men with alopecia androgenetica and effects of treatment with topical minoxidil: An exploratory study. *Int J Dermatol* 1988; 27 (Suppl.): 441–6.
3 Donk van der J, Passchier J, Dutree-Meulenberg ROGM *et al* Psychological characteristics of men with alopecia androgenetica and their modification. *Int J Dermatol* 1991 (in press).
4 Venning VA, Dawber RPR. Patterned androgenetic alopecia in women. *Br J Dermatol* 1987; 117 (Suppl.): 21–2.
5 Eckert P. Diffuse hair loss in women: the psychopathology of those who complain. *Acta Psychiat Scand* 1976, 53: 321–7.
6 Ludwig E Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. *Br J Dermatol* 1977; 97: 247–54.
7 Van Dam-Baggen R, Kraaimaat FW. *Handleiding bij de Inventarisatielijst Omgaan met Anderen.* Lisse: Swets & Zeitlinger, 1987; 11–34.
8 Sanders GJEM. *Het Gewone en het bijzondere van de Homosexuele Leefsituatie.* Deventer: Van Loghum Slaterus, 1977; 194–5.
9 Rosenberg M. *Society and the Adolescent Self-image.* Princeton, New Jersey: Princeton University Press, 1965; 16–18.
10 Luteijn F, Starren J, van Dijk H *Nederlandse Persoonlijkheids Vragenlijst, Handleiding.* Amsterdam: Swets & Zeitlinger, 1985
11 Appels A. *Voorlopige Handleiding bij de Delftse Vragenlijst.* Amsterdam: Swets & Zeitlinger, 1985
12 Secord PF, Jourard SM. The appraisal of Body-Cathexis: body cathexis and the self. *J Cons Psychol* 1953; 17: 343–7.
13 Tucker LA Internal structure, factor satisfaction, and reliability of the body-cathexis scale *Percept Motor Skill* 1981; 53: 891–6.
14 Balogun JA Reliability and construct validity of the Body Cathexis scale. *Percept Motor Skill* 1986, 62: 927–35
15 Dijkstra P. De Zelfbeoordelingsschaal vor Depressie van Zung. In: *Stemming en Ontstemming: Theorie en Praktijk bij de Diagnose en Dehandeling van Depressie.* (van Praag HM, Rooijmans HGM, eds) Amsterdam. De erven Bohn, 1974; 98–120.
16 Zung WWK. A self rating depression scale *Arch Gen Psychiat* 1965; 12: 63–70
17 van der Ploeg HM, Defares PB, Spielberger CD. *Handleiding bij de Zelf-Beoordelings-Vragenlijst, STAI-DY, een Nederlandstalige bewerking van de Spielberger State-Trait Anxiety Inventory.* Lisse: Swets & Zeitlinger, 1980.
18 van der Ploeg HM. Validatie van de Zelf-beoordelings-vragenlijst (een Nederlandstalige bewerking van de Spielberger State–trait Anxiety Inventory). *Ned Tijdschr Psych* 1980, 35: 243–9
19 Clayson DW, Maugham M. Blonde is beautiful: status and preference by hair color. Conference presentation. Rockey Mountain Psychological Association Convention, Phoenix, Arizona, 1976.
20 Vernis JS, Roll S. Primary and secondary male characteristics: The hairiness and large penis stereotypes. *Psychol Rep* 1970; 26: 123–6.
21 Moerman DE The meaning of baldness and implications for treatment. *Clin Dermatol* 1988, 6: 89–92.
22 Verbrugge L. Gender and health: an update on hypotheses and evidence. *J Health Soc Beh* 1985; 26: 156–82
23 Keep van PA. Isolation: the influence of skin disease on social relationships. *Int J Dermatol* 1974; 13: 227–31.
24 Jowett S, Ryan T. Skin disease and handicap. an analysis of the impact of skin conditions. *Soc Sci Med* 1985; 20: 425–9
25 Koning de EBG, Passchier J, Dekker FW. Psychological problems with hair loss in general practice and the treatment policies of general practitioners. *Psychol Rep* 1990; 67: 775–8.

British Journal of Dermatology (1991) 125, 253–255.

ADONIS 000709639100211U

The effect of oral indomethacin on psoriasis treated with the Ingram regime

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Accepted for publication 11 April 1991

Summary Fifty patients with psoriasis undergoing a standard Ingram regime were entered into a double-blind comparison of oral indomethacin and placebo. No significant differences were observed between the two groups in respect of disease activity or dithranol tolerance after 6 weeks.

There have been several reports of an exacerbation of psoriasis following the administration of indomethacin and other non-steroidal anti-inflammatory drugs (NSAID).[1–3] It has been postulated that inhibition of cyclo-oxygenase by NSAID might make psoriasis worse by diverting the metabolism of arachidonic acid through the lipoxygenase pathway and increasing the production of leukotrienes. It has also been suggested that administration of NSAID during dithranol therapy might be beneficial by reducing dithranol irritancy in the perilesional skin.[4]

There is therefore conflicting evidence regarding the influence of NSAID on psoriasis. We have undertaken a double-blind placebo-controlled study to determine whether indomethacin has any clinically relevant effect on psoriasis treated with the Ingram regime.

Methods

For the purposes of this study, we considered that a clinically relevant effect due to indomethacin should be greater than the standard deviation of the response of psoriasis to the Ingram regime. Using the nomogram of Altman,[5] a study of 50 patients should have a power of >90%. Thus 50 patients with chronic plaque psoriasis were entered into the study. Patients were treated on an out-patient basis with a standard Ingram regime of a coal-tar bath followed by exposure to UVB light in a suberythemal dose and then dithranol in Lassar's paste applied for 24 h. Dithranol was started at a concentration of 0·125% and increased at 2–3 day intervals through 0·25, 0·5, 1, and 1·5% to a maximum of 2% as tolerated.

Patients were randomly allocated either oral indomethacin 25 mg three times daily or placebo. Assessments were made before and after 6 weeks of treatment. An assessment of the body surface area (BSA) covered by psoriasis as a percentage of the total BSA was made using a Lund and Browder chart. In addition, two representative plaques were graded on a scale of 0–3 for erythema, scaling and induration. The mean of the two scores was taken as the plaque severity score, giving a maximum possible score of 9.

The differences between the two treatment groups were analysed by Wilcoxon rank pairs tests for non-parametric data and *t*-tests for parametric data.

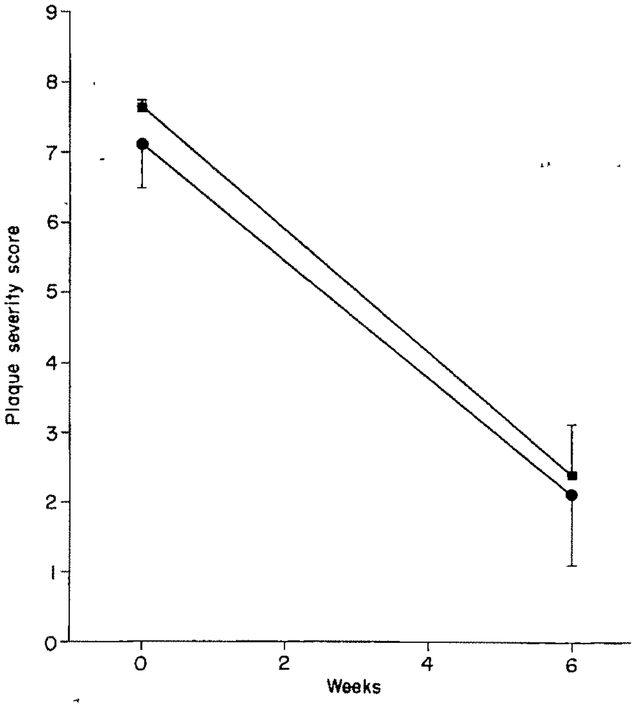

Figure 1. Mean plaque severity scores with 95% confidence intervals for indomethacin (●)- and placebo (■)-treated patients.

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Results

Forty-one patients completed the study, 19 in the indomethacin group (14 male and five female; mean age 41 years, range 17–78 years) and 22 in the placebo group (16 male and eight female, mean age 39 years, range 18–75 years). There were no significant differences between the two groups at the start of treatment in respect of age, sex ratio, plaque severity scores and BSA of psoriasis. Eight patients failed to complete the 6 weeks of treatment and were lost to follow-up, and one patient in the placebo group stopped her medication because of dyspepsia and was subsequently lost to follow-up.

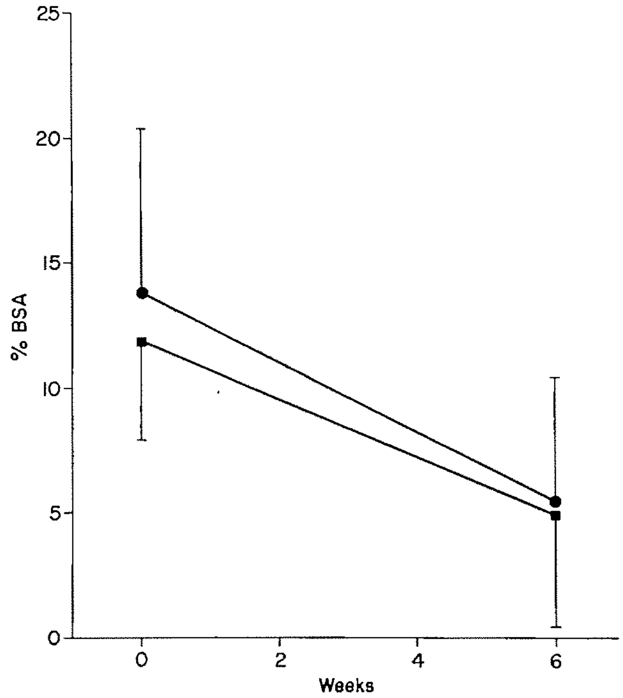

Figure 2. Mean per cent BSA of psoriasis with 95% confidence intervals for indomethacin (●)- and placebo (■)-treated patients.

Both the indomethacin and placebo groups had highly significant reductions in plaque severity scores at 6 weeks ($P<0{\cdot}00001$ and $P<0{\cdot}00001$, respectively) (Fig. 1). However, there was no significant difference between the two groups (70% and 68% for indomethacin and placebo, respectively).

Similarly, there were statistically significant reductions in BSA in both indomethacin and placebo groups at 6 weeks ($P<0{\cdot}001$ and $P<0{\cdot}01$, respectively), but the difference between the groups was not statistically significant (58% and 42% for indomethacin and placebo, respectively) (Fig. 2).

Although the majority of patients in both groups had a reduction in BSA of psoriasis by 6 weeks, a small proportion in each group had become worse (two out of 19 of the indomethacin group and four out of 22 of the placebo group). Of these, one patient in the indomethacin group and two in the placebo group had increases in BSA of psoriasis of over 50% (Fig. 3).

There were no significant differences in tolerance of dithranol between the indomethacin and placebo groups, as indicated by the final dithranol concentrations achieved at 6 weeks (Fig. 4).

Discussion

Several patients have been reported in whom deterioration of psoriasis followed use of NSAID. Katayama and

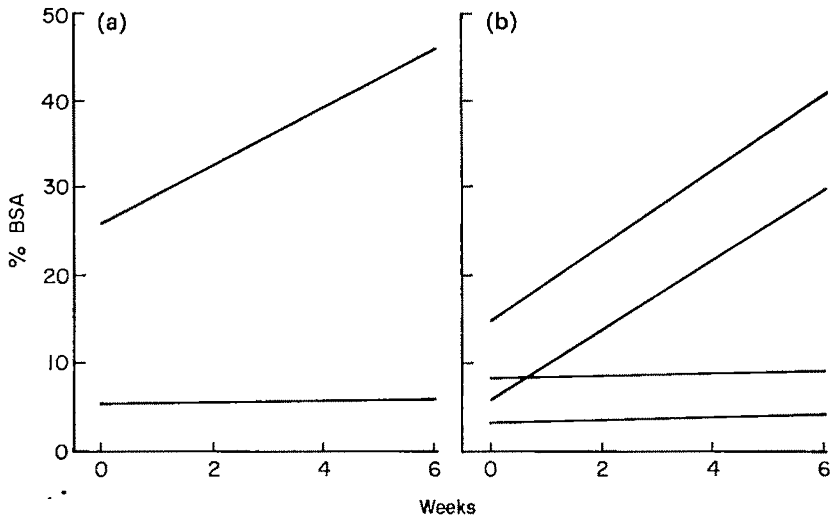

Figure 3. Individual changes in per cent BSA of psoriasis for patients in whom psoriasis worsened during the Ingram regime. (a) indomethacin (b) placebo.

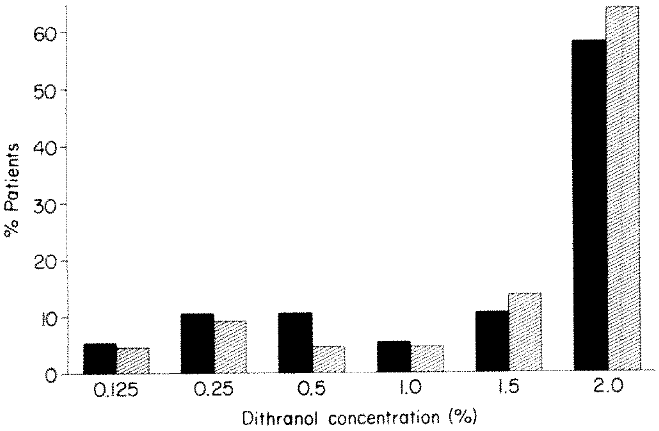

Figure 4. Final dithranol concentrations tolerated by indomethacin (■)- and placebo (▨)-treated patients.

Kawada[1] reported worsening of psoriasis in six patients taking oral indomethacin. Reshad *et al.*[2] subsequently reported two patients who developed generalized pustular psoriasis, one following phenylbutazone and the other following oxyphenbutazone. The authors urged caution when considering these drugs for patients with psoriasis.

Other authors have suggested a possible beneficial role for NSAID in psoriasis. In a preliminary study of 20 psoriatics undergoing dithranol therapy, Shall and Marks[4] found a trend towards faster clearing and better dithranol tolerance with oral indomethacin compared to placebo after up to 28 days of treatment, but the differences were not statistically significant. In a smaller double-blind placebo-controlled crossover study of five patients with psoriasis for whom all active treatment was discontinued, two of the patients deteriorated while taking indomethacin.[3]

We have shown in a larger study that indomethacin in a standard dose of 75 mg daily has no significant harmful effect on psoriasis in patients treated with the Ingram regime, and does not influence their tolerance to dithranol.

It is possible that patients attending a dermatology clinic for treatment of their psoriasis represent a selected group whose response to indomethacin is different from those whose psoriasis is in remission. However, patients with psoriasis attending a dermatology clinic will tend to be in an active phase and it seems likely that such patients would be more responsive to any pro-inflammatory pharmacological action of indomethacin.

It is possible that an idiosyncratic reaction to a NSAID could worsen psoriasis, but this study excludes a clinically significant pharmacological effect of indomethacin. The degree of variation in response to the Ingram regime observed in this study was large for both indomethacin and placebo groups. The observation that significant worsening of psoriasis can occur in patients whether they are receiving indomethacin or not, calls into question the significance of anecdotal reports of worsening of psoriasis in patients taking such a widely prescribed group of drugs as the NSAID. We consider that there are no grounds to withhold indomethacin from patients with psoriasis and co-existing arthritis.

References

1 Katayama H, Kawada A. Exacerbation of psoriasis induced by indomethacin. *J Dermatol* 1981; **8**: 323–7.
2 Reshad H, Hargreaves GK, Vickers CFH. Generalized pustular psoriasis precipitated by phenylbutazone and oxyphenbutazone. *Br J Dermatol* 1983; **109**: 111–3.
3 Powles AV, Griffiths CEM, Seifert MH, Fry L. Exacerbations of psoriasis by indomethacin. *Br J Dermatol* 1987; **117**: 799–800.
4 Shall L, Marks R. The effects of concomitant administration of oral indomethacin with topical dithranol treatment in psoriasis. *Br J Dermatol* 1987; **116**: 739–44.
5 Altman DG. How large a sample? In: *Statistics in Practice* (Altman DG, Core SM, eds). London: British Medical Association, 1982: 6–8.

British Journal of Dermatology (1991) 125, 256–259. ADONIS 0007096391002128

Ablation of whiteheads by cautery under topical anaesthesia

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Accepted for publication 9 April 1991

Summary A simple treatment is described for the ablation of whiteheads which are 'marsupialized' by light cautery under local anaesthesia with EMLA® cream.

The treatment of acne has seen major advances in recent decades.[1–3] However, a number of patients have large whiteheads (2·0 mm in diameter) which are rarely if ever helped by conventional therapy with oral or topical antimicrobials or retinoids. Standard dermatology reference books and specialized textbooks[4–6] give little or no indication as to the best way to treat these lesions. Mechanical removal of blackheads is recognized as useful and comedone extractors of the Unna, Scalfield, Lawton and Schomberg types have been used. Suction has also been reported to be of help.[7,8]

Whiteheads, when large and numerous, are disfiguring and difficult, if not impossible, to camouflage using make-up. Such lesions may cover a large area of the face, especially the cheeks, and surgical techniques would require multiple injections of local anaesthesia.

We have investigated as to how well patients tolerate light cautery to their whiteheads using EMLA® cream as a local anaesthetic, and as to how effective this treatment is.

EMLA® cream 5% is a eutectic mixture of lignocaine 25 mg/g and prilocaine 25 mg/g and is an effective local anaesthetic when applied to the skin and occluded with polythene. It has been used for anaesthesia in the treatment of port wine stains and other skin lesions with variable success.[9,10]

Methods

Fourteen patients (11 female, three male) aged 16–66 years, with large whiteheads (2–3 mm) were treated. Their details are summarized in Table 1. All had received long-term therapy including oral and topical antimicrobials, benzoyl peroxide (5–10%) and topical tretinoin (0·05%). The oral antibiotics had been given in doses of 1·0 g/day of tetracycline and/or 200 mg/day of minocycline. Seven patients had received oral isotretinoin. None of these agents had any effect on the large whiteheads.

EMLA® cream was liberally applied to the area affected by the whiteheads, either by the patient or doctor and firmly occluded with self-adhesive polythene dressing. The patient then waited for 1–3 h and the EMLA® was then removed with a dry swab. Cautery (Thackray 3VAC) was used with a light touch sufficient to ablate small lesions and puncture or 'marsupialize' the larger ones. Treatment was commenced at the centre of the area of anaesthesia, working peripherally. In each patient, one whitehead outside the field of EMLA® anaesthesia was also treated to assess the pain produced by the cautery.

On each patient a test area of 5–15 lesions was treated initially and the cosmetic result reviewed a fortnight later. The total number of lesions treated per patient varied from 10 to 230 (mean 56·9). The time taken for the cautery treatment was 5–20 min depending on the number of lesions treated. The total number of treatments each patient received varied from 1 to 5 (mean 2·4). At the completion of each treatment, the patient was asked to rate the pain experienced during the procedure using an aural/verbal analogue scale of 0 (no pain)–10 (agony/torture).

The procedure was repeated at 2-week intervals until cosmetically troublesome whiteheads had resolved. Photographs were taken before treatment, after 2 weeks and several months (usually 6) following treatment.

Results

Fourteen patients received a total of 34 treatments (mean 2·4 per patient). A total of 805 lesions were treated.

All patients considered the treatment to be beneficial. This was confirmed objectively by photography (Figs 1

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Table 1. Patient characteristics

PT	Sex	Age	Total number of treatments	Total number of lesions treated	EMLA application time (min)	Pain score
					(1st) (2nd) (3rd) (4th) (5th)	(1st) (2nd) (3rd)
1	M	16	2	45	(60) (60)	(2) (3)
2	F	41	3	63	(135) (135) (130)	(1) (2) (1)
3	F	50	2	29	(130) (135)	(1) (1)
4	F	66	5	49	(120) (100) (85) (60) (120)	(1) (1) (1) (1) (1)
5	F	30	3	230	(110) (150) (90)	(1) (2) (1)
6	F	40	4	53	(60) (90) (120) (180)	(4) (1) (0) (2)
7	F	22	3	54	(105) (110) (120)	(0) (0) (0)
8	F	29	2	18	(60) (120)	(5) (1)
9	F	28	2	67	(100) (120)	(2) (1)
10	F	23	1	10	(90)	(1)
11	M	19	1	10	(100)	(4)
12	F	21	3	69	(90) (90) (120)	(3) (1) (1)
13	F	19	2	95	(90) (105)	(3) (2)
14	M	18	1	13	(90)	(3)

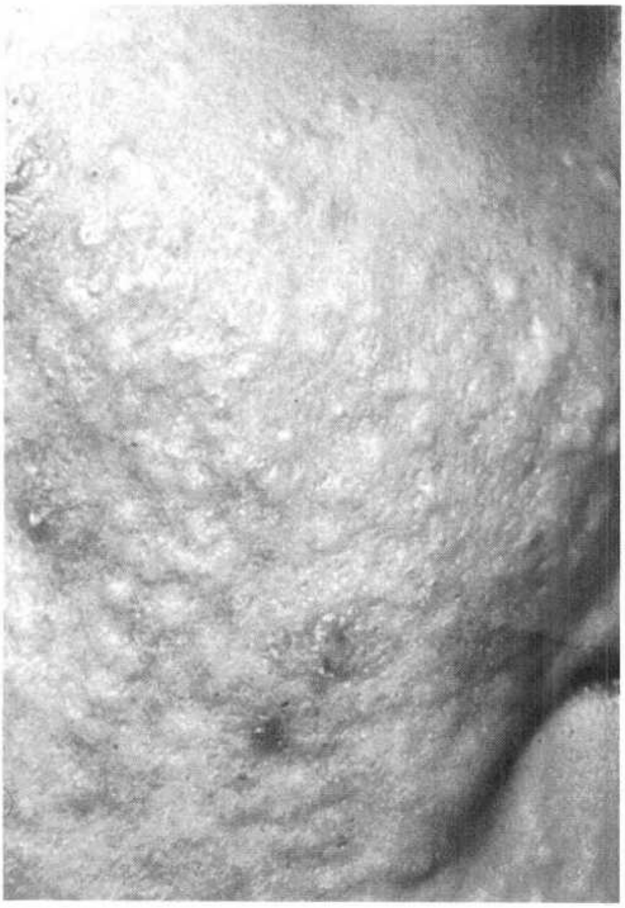

Figure 1. 22-year-old woman with 6-year history of severe acne, pretreatment.

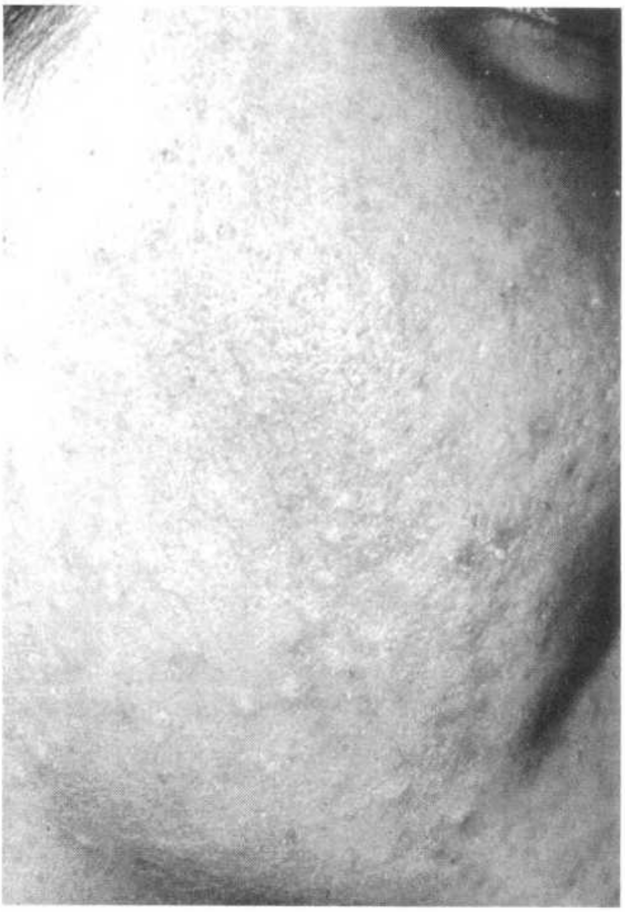

Figure 2. Same patient 2 months after most of the cheek had been treated. This patient received a further therapy which cleared all but three macrocomedones.

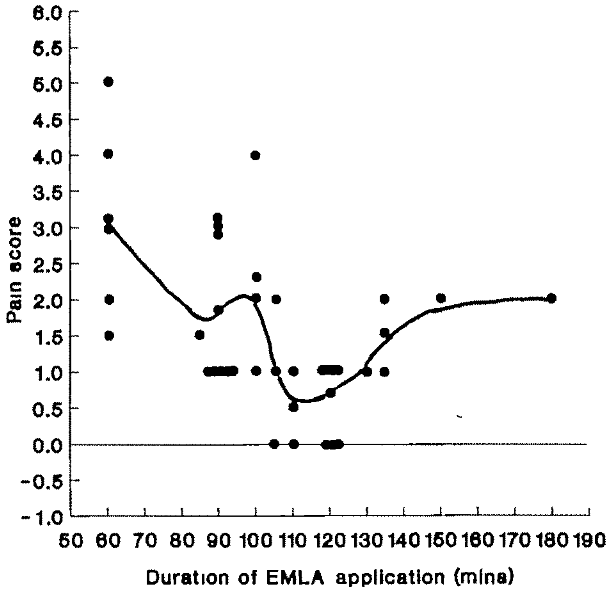

Figure 3. Pain score versus duration of application of EMLA® to the skin prior to cautery treatment. —●—, Mean pain score.

and 2) and by lesion counts. Of the 805 whiteheads treated, more than 95% had cleared.

Pain scores varied with the duration of application of EMLA® to the skin (Fig. 3). The lowest scores were recorded for application times of between 1 h 45 min and 2 hr 10 min. Pain scores were greatest up to 1 hr 40 min and increased again with application times of longer than 2 h 15 min. Pain scores recorded for the single lesion treated without anaesthesia varied from 6 to 9 using the pain scale described. All patients considered the anaesthesia effective and none found the procedure very painful. Post-operative pain was not a problem and no patient required any analgesia. The treated areas showed the effect of minor heat damage, but healed quickly after 4–7 days and without any complication. Follow-up of 6–14 months (mean 7·6, median 7) showed no recurrence, scarring or pigmentation of the treated areas.

Discussion

Our study demonstrates the success of this technique in treating large whiteheads and that EMLA® applied to the skin under polythene occlusion is a reliable anaesthetic. The anaesthetic response varied with the duration of application of EMLA®, maximal anaesthesia being recorded at 2 h in our adult patients. This is in contrast to the manufacturers' instructions of 1 h on the limbs of children prior to venepuncture, and may explain the variable effect in adult patients in a study of port wine stains.[9] Up to 100 whiteheads could be treated at one time in our patients. There was transient blanching of the skin produced by EMLA® cream, making visualization of the whiteheads difficult unless careful lighting and palpation were used. However, this blanching appeared to be a useful indication that the EMLA® cream had penetrated the skin.

Large whiteheads are not only a cosmetic problem, but frequently develop into inflamed lesions. They can last for years and there is no universally accepted treatment. Long-term antibiotics given in adequate doses for papulo-pustular acne are of no help in the treatment of whiteheads and neither are topical benzoyl peroxide or retinoic acid. Even oral isotretinoin, given to half of our patients, was of no benefit in the treatment of their whiteheads. Before developing this technique we treated whiteheads by attempting to identify the eccentrically placed punctum, which was opened with a fine needle and the contents extracted with a comedone extractor and the duct wall touched with phenol. This technique was unsatisfactory and recurrence was common. For this reason we did not perform a comparative study. Sebo-suction using suction of up to 30 inches of mercury was reported in the U.S.A. in 1970 and 1983.[7,8] but there have been no further publications on this technique.

We considered comparing our technique on one side of the face with 'sham' cautery on the other side. However, the immediate effect of the procedure in producing minute areas of heat damage would make such a study difficult.

We believe that we have demonstrated for the first time a beneficial treatment for large whiteheads. The cautery technique itself is quick and simple to perform and lesion counts and photography clearly confirmed its success. We therefore recommend cautery under EMLA® anaesthesia for the treatment of patients with large whiteheads.

Acknowledgements

The authors would like to thank Mrs Susan Pierce for typing the manuscript.

References

1 Pedace FJ, Stoughton R Topical retinoic acid in the treatment of acne. *Br J Dermatol* 1971, **84**: 465–9

2 Cunliffe WJ, Clayden AD, Gould D, Simpson NB Acne vulgaris: its aetiology and treatment. A review. *Clin Exp Dermatol* 1981, **6**: 461–9.

3 Farrell LN, Strauss JS, Stranieri AM. The treatment of severe cystic acne with 13-*cis*-retinoic acid. Evaluation of sebum production and clinical response in multiple dose trial. *J Am Acad Dermatol* 1980; **3:** 602–11.

4 Cunliffe WJ, Ebling FJG. The sebaceous gland. In: *Textbook of Dermatology* (Rook A, Wilkinson DS, Ebling FJG *et al* eds). London: Blackwell Scientific Publications, 1986; 128.

5 Strauss JS. Sebaceous glands. In: *Dermatology in General Medicine* (Eisen AZ, Wolff K, Freedberg I, Austin KF, eds). New York: McGraw-Hill, 1987; 673.

6 Cunliffe WJ. Treatment of acne. In: *Acne* (Cunliffe WJ, eds). London: Martin Dunitz, 1989; 279.

7 Potts LW. Comedone extraction by aspiration—new therapy for acne. *Cutis* 1970; **6:** 669–70.

8 Pierce HE. Sebo-suction—an effective adjunctive treatment for comedone acne. *J Dermatol Surg Oncol* 1983; **9:** 955.

9 Lanigan SW, Cotterill JA. Use of a lignocaine–prilocaine cream as an analgesic in dye laser treatment of port-wine stains. *Lasers Med Science* 1987; **2:** 87–9.

10 Juhlin L, Evers H, Broberg F. A lignocaine–prilocaine cream for superficial skin surgery and painful lesions. *Acta Derm Venereol (Stockh)* 1980; **60:** 544–6.

British Journal of Dermatology (1991) 125, 260–262.

ADONIS 00070963900213M

Successful 2-week treatment with terbinafine (Lamisil®) for moccasin tinea pedis and tinea manuum

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Accepted for publication 12 April 1991

Summary A new orally active antifungal agent, terbinafine, was used in the treatment of tinea pedis ('dry type' or moccasin type) and tinea manuum. Fifty-three adults over the age of 16 years with fungal infections of the feet and/or hands were treated with either oral terbinafine, 250 mg, or placebo, once daily for 2 weeks. The diagnosis of fungal infection was confirmed by examination of skin scrapings by microscopy and culture. Of these, 28 patients were evaluable for efficacy. At 8 weeks, 12 out of 14 (86%) patients who received terbinafine were mycologically negative (microscopy and culture) compared to one out of 14 (7%) patients on placebo ($P<0{\cdot}001$, Fishers exact test, one-sided). At the end of the study 71% of patients in the terbinafine group were judged to have received effective therapy compared to 0% in the placebo group ($P<0{\cdot}001$). Terbinafine was well tolerated, and more side-effects were seen in the placebo group.

The dermatophyte fungi that cause disease in man all invade the stratum corneum or keratinized tissues derived from the epidermis, such as hair or nails. The spectrum of clinical disease and the responses to treatment vary considerably with different species and sites of infection.[1] Generally most infections respond satisfactorily to topical antifungal agents, but the present treatment of more severe fungal infections of the hands and feet (i.e. 'dry type' or moccasin type) usually caused by *Trichophyton rubrum*, is very unsatisfactory. These infections are resistant to therapy and do not respond as well to topical antifungal agents, or even treatment with the newer azole drugs. Typically, treatment with an oral antifungal (e.g. griseofulvin 1 g daily for 3–6 months) is required, and even then there is a poor response rate and a high relapse rate.[2] Unsatisfactory results have also been obtained with the oral imidazole ketoconazole.[2] The risk of hepatotoxicity associated with ketoconazole also limits its use in such infections. Itraconazole is an oral triazole which has been shown to be more active than griseofulvin or ketoconazole,[3] but treatment in moccasin type infections should continue for 30 days. The often poor therapeutic response to these antifungal drugs is probably due to their fungistatic action against the dermatophyte fungi which cause these infections.

Terbinafine (Lamisil®) is a synthetic antimycotic agent from a new class of compounds, the allylamines. Studies *in vitro* have shown terbinafine to be fungicidal against dermatophytes and a number of other moulds and yeasts.[4,5] A clinical study in moccasin tinea pedis has shown that oral terbinafine was more effective than griseofulvin when used for 6 weeks.[6] Because of its fungicidal activity it was thought that even shorter durations of therapy may be possible.

Methods

Fifty-three patients over the age of 16 years with clinically diagnosed fungal infections of the hands and/or feet were entered into this double-blind, parallel group study comparing terbinafine 250 mg once daily for 2 weeks with placebo. Ethical Committee approval was obtained for the trial and all participants gave their informed consent. The patients were all suffering from a fungal infection that had spread beyond the toe or finger clefts on to the sole and/or dorsum of the foot and palms of the hands. This diagnosis was confirmed by mycological culture of skin material. Patients whose cultures were subsequently negative were classified as delayed exclusions and were omitted from the efficacy analysis. All patients entered were, however, included in the analysis of safety. Clinical assessments were made at baseline and at 2, 4, 6 and 8 weeks. Signs and symptoms including erythema, scaling, vesiculation, pustules, exudation, crusting and pruritus were rated on a scale of 0–3 (absent–severe). The location and extent of the infection was also recorded diagrammatically. Mycological assessments were made at baseline and at 2, 4, 6 and

8 weeks. Standard haematological and biochemical screens were performed weekly during the treatment phase.

Results

Of the 53 patients, 20 were classified as delayed exclusions due to negative baseline microscopy and/or cultures. Three patients were lost to follow-up and two violated the protocol significantly (one patient used a corticosteroid preparation during the study period and one patient had tinea corporis). Twenty-eight patients could therefore be evaluated for efficacy, 14 in the terbinafine group and 14 in the placebo group. The mycology results are shown in Table 1 and Figure 1.

At 2 weeks (end of treatment) there was no statistical difference between terbinafine and placebo-treated patients. However, at 8 weeks (6 weeks after completion of therapy) 12 out of 14 (86%) patients who received terbinafine were mycologically cured (negative microscopy and culture) compared to one out of 14 (7%) who received placebo, a statistically significant difference ($P<0{\cdot}001$, Fishers exact test, one-sided).

The mean reduction in the symptoms and signs of infection was significantly greater in the terbinafine group than in the placebo group, both at the end of treatment (2 weeks) and at week 8 (Mann–Whitney *U*-test, $P<0{\cdot}05$ and $P<0{\cdot}001$, respectively). The mean reductions in symptom scores are shown in Table 2 and Figure 2.

Table 1. Number of patients with negative mycology (microscopy and culture) (percentage in parentheses)

	Terbinafine (%)	Placebo (%)
Week 2	3/13 (23)	1/14 (7)
Week 4	9/14 (64)	1/14 (7)
Week 6	10/14 (71)	2/14 (14)
Week 8	12/14 (86)	1/14 (7)

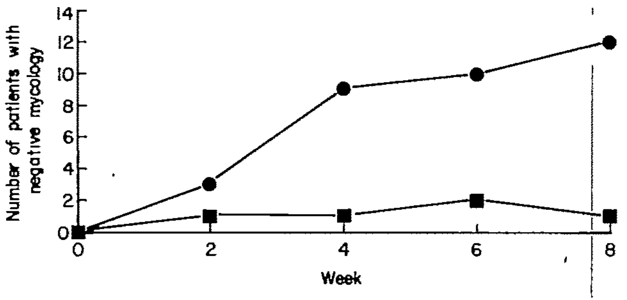

Figure 1. Mycological assessment of patients. ●, Lamisil; ■, placebo.

Table 2. Summary of sums of clinical scores

	Terbinafine	Placebo
Baseline	6·7	5·6
Week 2	3·1	3·9
Week 4	2·8	4·0
Week 6	2·3	4·3
Week 8	1 6	4·2

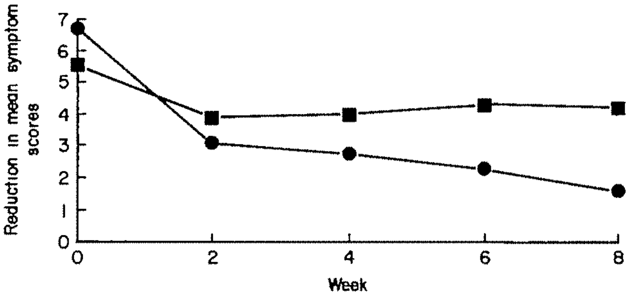

Figure 2. Clinical assessment of patients. ●, Lamisil; ■, placebo.

The proportion of patients judged to have received effective therapy was determined by combining the mycological and clinical results. At the follow-up assessment at week 8, 10 out of 14 (71%) patients in the terbinafine group compared to 0 out of 14 in the placebo group were classified as being effectively treated (Fishers exact test, one-sided, $P<0{\cdot}001$). The proportion of patients effectively treated is shown in Table 3.

There were no clinically significant changes in the haematological, hepatic or renal blood tests. Emergent symptoms were recorded in eight patients receiving terbinafine compared to 14 receiving placebo. Five were thought to be probably or possibly related to treatment. Two were in the terbinafine group (desquamation of skin, mild diarrhoea) and three were in the placebo group (back pain, diarrhoea, abdominal discomfort). One patient on placebo was withdrawn from the study due to moderate bilateral inflamed eyes that was thought to be unlikely to be related to treatment.

Discussion

Infections of the hands and feet with *T. rubrum* are the most difficult dermatophyte infections to clear. In this study 26 out of 28 (93%) of the cases were due to this organism, the remaining two being one patient with

Table 3. Proportion of patients effectively treated (percentage in parentheses)

	Terbinafine (%)	Placebo (%)
Week 2	1/13 (8)	0/14 (0)
Week 4	6/14 (43)	0/14 (0)
Week 6	6/14 (43)	0/14 (0)
Week 8	10/14 (71)	0/14 (0)

Epidermophyton floccosum in the terbinafine group who responded to treatment and one patient with *T. mentagrophytes* in the placebo group. Forty-three per cent of the patients had already failed to improve with previous therapy. Terbinafine achieved an 86% mycological cure following only a 2-week course of therapy and the drug was well tolerated. This high efficacy following a short duration of treatment is likely to be due to the fungicidal activity of the compound and its rapid delivery to the target tissues involved in dermatophytosis.

Acknowledgments

We thank Sandoz U.K. who supplied the drug, D.A.R. Limited, Cambridge for organizing the trial and for the statistical analysis, and the following colleagues: Dr R.Dawber, Lt. Col. K.Freeman, Dr B.R.Allen, Dr D.M.Williamson, Dr D.Gould, Dr M.Goodfield and the late Dr L.Puttick for allowing us to present the results for their patients.

References

1 Roberts SOB. Treatment of the superficial and subutaneous mycoses. In: *Antifungal Chemotherapy* (Speller DCE, ed) Chichester: John Wiley & Sons, 255–83.
2 Lambert DR, Siegle RJ, Camisa MD. Griseofulvin and ketoconazole in the treatment of dermatophyte infections *Int J Dermatol* 1989; 28: 300–4.
3 Saul A, Bonifaz A. Itraconazole in common dermatophyte infections of the skin: Fixed treatment schedules *J Am Acad Dermatol* 1990; 23(Suppl): 554–8.
4 Petranyi G, Meingassner JG, Mieth H. Antifungal activity of the allylamine derivative terbinafine *in vitro*. *Antimicrob Agents Chemother* 1987, 31: 1365–8.
5 Clayton YM *In vitro* activity of terbinafine. *Clin Exp Dermatol* 1989; 14: 101–3.
6 Savin R. Successful treatment of chronic tinea pedis (mocassin) type with terbinafine (LAMISIL). *Clin Exp Dermatol* 14: 116–19.

British Journal of Dermatology (1991) **125**, 263–271. ADONIS 000709639100214Z

Lichen planus pemphigoides: its relationship to bullous pemphigoid

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Summary Clinical and immunopathological studies of three patients with lichen planus pemphigoides (LPP) were carried out to investigate the relationship between LPP and bullous pemphigoid (BP) and to determine whether the antigen in LPP is the classical BP antigen. LPP is usually considered to be the coexistence of lichen planus with BP. The bullae in LPP were subepidermal and indistinguishable from BP. Indirect immunofluorescence demonstrated antibody binding to the epidermal surface of 1 M NaCl-split skin and mucosae, as in BP. The tissue distribution of the LPP antigen mirrored the distribution of BP in stratified squamous epithelia but was absent from transitional epithelia (pig bladder). Immunoelectron microscopy, both direct (two cases) and indirect (one case), showed binding to the lamina lucida as with BP antigen. Western blotting of epidermal extracts using the patients' sera showed that instead of reacting with the classical bullous pemphigoid antigen (220 kDa in our series), the antisera reacted with a unique band of 200 kDa in addition to the band of 180 kDa found as a minor antigen in bullous pemphigoid, but more commonly in pemphigoid gestationis. The relationship between these antigens awaits molecular characterization. These findings suggest that the target antigen in LPP may be unique.

There has been controversy regarding the nature of lichen planus pemphigoides (LPP) ever since Kaposi first coined the term 'lichen ruber pemphigoides' in 1892[1] to describe a case of typical lichen planus (LP) complicated by a widespread bullous eruption. Two types of bullous LP were recognized by Schreiner,[2] the first having small vesicles occurring only in pre-existing LP papules whilst the second type had bullae as a dominant feature arising on clinically normal as well as on lesional skin and more suggestive of bullous pemphigoid (BP). Recent studies support this distinction[3] and immunofluorescence techniques have led to a further delineation of the two groups. On direct immunofluorescence, linear deposition of C3 and IgG along the basement membrane zone (BMZ) has been described in LPP[3-12] and more than 50% of cases are reported to have circulating IgG anti-BMZ antibodies.[13] These findings have led most authors to conclude that LPP is the coexistence of two separate diseases, LP and BP.[4,5,7,11] It has been proposed that damage to the basal cells in LP unmasks hidden antigenic determinants, or creates new antigens, leading to autoantibody formation and the induction of BP.[4]

The debate now centres on whether the bullous eruption of LPP is true BP or a BP-like disorder with autoantibodies directed against a BMZ antigen distinct from the BP antigen.[3] In two cases of LPP immunoelectron microscopy has shown deposits in the lamina lucida, as in BP.[9,14] The circulating antibody in one case of drug-induced LPP was found to immunoprecipitate the BP antigen[15] while in another case of LPP it failed to do so.[16] The latter study indicates that the target antigen in LPP may differ from the BP antigen.

We have studied three patients with LPP using immunofluorescence, immunoelectron microscopy and immunoblotting techniques in order to define further the immunopathology of this disorder and the relationship of the target antigen to classical BP.

Case reports

Case 1

A male Caucasian aged 74 years, had a 40-year history

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This work was presented in part at the annual meeting of the British Society for Investigative Dermatology, Manchester, U.K., September 1988.

of hypertrophic LP of the shins treated by repeated excision and grafting (three times in 1949 and once in 1960). He presented with an enlarging, hyperkeratotic fissured area at the lower end of a scar on the left shin in association with typical LP of the trunk, limbs, hands and feet. An initial biopsy of the shin lesion showed only hypertrophic LP with basal-cell liquefaction and a dense upper dermal infiltrate of lymphocytes. However, the epidermis was hyperplastic with an increased stratum granulosum and stratum corneum. A repeat biopsy showed a well-differentiated squamous cell carcinoma which was treated by wide excision and grafting. Five days post-operatively he developed a pruritic erythematous eruption on the hands and subsequently an urticated erythematous eruption appeared on the trunk and limbs, with tense bullae in these areas as well as on normal skin.

A skin biopsy taken from an urticated erythematous lesion on the thigh showed a superficial dermal infiltrate of eosinophils and mononuclear inflammatory cells, with perivascular accentuation. There was oedema and degeneration beneath the basal layer but no fully formed bullae. These features were thought to be typical of bullous pemphigoid. Direct immunofluorescence of perilesional thigh skin showed linear deposits of IgG and C3 at the BMZ. Indirect immunofluorescence showed a circulating IgG autoantibody binding to the epidermal BMZ at a titre of 1/160.

The blisters cleared rapidly after oral prednisolone 30 mg/day was started and the dose was reduced and azathioprine added for its steroid-sparing effect. He is now in remission and off all treatment.

Case 2

A male Caucasian aged 58 years, presented with a 6-month history of an itchy papular eruption beginning on the wrists and neck and gradually spreading to involve the trunk and limbs. He had a past history of an episode of pompholyx 10 years previously. He had had a laminectomy and suffered from cramps. His medication was diclofenac, dextropropoxyphene with paracetamol, and quinine. On examination he had generalized and clinically typical LP. There was no involvement of the mucous membranes. He was advised to stop his quinine and was treated with topical steroids. Despite discontinuing quinine there was no resolution of his lichen planus and 1 month later he was admitted with a new blistering eruption distributed mainly on the trunk. Some of the blisters occurred on lesions of typical LP and others on an erythematous urticated base. The clinical picture was not thought to be that of a lichenoid drug eruption.

Skin biopsies were taken from two areas. The first, taken from an area of clinical LP, showed the classical changes of this disease with colloid body formation, basal layer degeneration and an upper dermal lymphocytic infiltrate. A biopsy from a blistered area showed marked oedema of the papillary dermis and an inflammatory infiltrate in the upper dermis comprising lymphocytes and eosinophils. Small subepidermal bullae were present. Direct immunofluorescence of uninvolved forearm skin and perilesional skin showed linear deposition of C3 along the BMZ. Circulating autoantibodies were found to the BMZ at a titre of 1/80.

He was treated with prednisolone and required 80 mg daily before his eruption came under control. The dose was subsequently reduced to 7·5 mg daily. Nine months after presentation, with no further exposure to quinine, he developed further itchy urticated weals on the back and thighs and the dose of prednisolone was increased to 20 mg daily. After some response the dose has since been reduced to 15 mg daily.

Case 3

A 44-year-old West Indian man, presented with a 6-week history of itchy papules which appeared initially on the wrists and subsequently became generalized. Four weeks after the onset of the rash he developed blisters both in association with these lesions and on normal-appearing skin. On examination there was generalized LP with involvement of the palate appearing as a network of white streaks. Tense blisters were present, especially on the ankles and wrists and later on the palate.

A skin biopsy taken from a blister arising on a lesion of LP on the forearm revealed subepidermal clefts immediately adjacent to an area showing the typical changes of LP. A second biopsy of a blister showed a subepidermal bulla with a mild chronic inflammatory cell infiltrate composed predominantly of lymphocytes in the dermis. Direct immunofluorescence of perilesional skin from the forearm showed linear binding of IgG and C3 along the BMZ. Indirect immunofluorescence studies were initially positive but subsequently negative.

He was treated with prednisolone 30 mg daily. Two weeks later he was admitted with colicky abdominal pain and on endoscopy was found to have a pyloric canal ulcer. He was treated with ranitidine 50 mg b.d. His skin rash cleared although he had marked post-inflammatory hyperpigmentation. Steroids were withdrawn completely over a period of 5 months. He subsequently developed hypertrophic LP on his shins which was treated with topical steroids.

Methods

Tissue specimens

In addition to skin biopsies performed for diagnostic purposes, the following specimens were taken from all three patients for direct immunofluorescence studies: 4-mm punch biopsies of normal forearm skin, mucosa of the lower lip, a suction blister of the forearm and a biopsy of bulbar conjunctiva. These studies were performed with informed consent and ethical clearance. Conjunctival biopsies were performed, by a trained ophthalmologist, as previous studies had shown that conjunctiva gave the highest sensitivity for direct immunofluorescence in studies of bullous pemphigoid.[17]

The following tissues were obtained for indirect immunofluorescence studies from patients with no evidence of skin disease or of malignancy: normal human ear skin, human forearm skin, oral mucosa, vaginal mucosa and conjunctiva. Two full-term placentae were obtained following vaginal delivery from patients who had had a normal pregnancy. Previous studies had shown that pig bladder distinguished between the target antigens of bullous pemphigoid and linear IgA disease,[18] so in addition frozen samples of fresh adult pig bladder were used.

All the tissues for immunofluorescence were mounted in Ames OCT compound (Ames Co), snap frozen in liquid nitrogen and stored at −70°C.

Skin and mucosal separation technique

Normal human ear skin and vaginal mucosa were trimmed, washed in PBS and cut into 1 mm pieces. The tissue was then incubated for periods of 24–72 h in freshly prepared 1 M NaCl solution at 4°C or 13°C with continuous agitation to separate the epidermis from the dermis. Once separation occurred, the tissue was then embedded in OCT compound, snap frozen in liquid nitrogen and stored at −70°C.[19]

Sera

Serum was obtained from all three patients with LPP as well as from three patients with LP, six positive BP controls (one well-characterized serum, a gift from Dr John Stanley, NIH, Bethesda, U.S.A.) and from three normal volunteers. All samples were stored at −20°C. Because limited amounts of stored sera were available, patients were bled for detailed immunofluorescence and blotting studies at return visits, at which times Patients 1 and 2 had no active blisters. All LPP sera were used for indirect immunofluorescence and immunoelectron microscopy and immunoblotting against both epidermal and dermal extracts. The control sera of bullous pemphigoid, lichen planus and normal serum were also immunoblotted.

Immunofluorescence studies

Direct immunofluorescence. A standard direct immunofluorescence (IF) technique was used to stain 6-μm cryostat-cut sections of tissue. Slides were incubated with FITC-labelled anti-human IgG, IgA, IgM and C3 (Dako F/P 2.3M).[20]

Indirect immunofluorescence. Sections were incubated with patients' serum at the following dilutions: neat, 1/2 and 1/5, prior to incubation with FITC-labelled anti-human IgG (Dako F/P 2.3M). The titres found by serial dilution were Patient 1, 1/160; Patient 2, 1/80; Patient 3, negative.

Immunoelectron microscopy

Direct. A 4-mm punch biopsy from the clinically uninvolved skin of the forearm was cut in half. One half was processed for immunofluorescence while the other was used for immunoelectron microscopy. This tissue was sliced into thin pieces which were divided into two groups. The slices in the first group were incubated with horseradish peroxidase (HPO)-labelled rabbit anti-human IgG (Dako F/P ratio 2:3 M). The slices in the second group were incubated with unrelated HPO-labelled immunoglobulin (negative control). Negative controls also included normal human skin incubated with HPO-labelled IgG. The slices were then processed for electron microscopy by a previously described method.[21] Indirect immunoelectron microscopy was also performed by incubation of the autoantisera with normal human trunk skin prior to processing. Control samples of normal forearm skin were used for direct immunoelectron microscopy and normal human serum for the indirect immunoelectron microscopy.

Immunoblotting

Tissue preparation. Extracts of human skin basement membrane zones (BMZ) were prepared by incubating the skin in 1 M NaCl containing 50 μm phenylmethylsulphonylfluoride (PMSF) at 4°C for 72 h, then removing the epidermis. A basement membrane preparation was made from both the epidermal and dermal fragments by incubating them separately in 8 M urea containing 0·3 M 2-mercaptoethanol and 50 μm PMSF overnight. Dermal and epidermal extracts were then run separately on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) prior to immunoblotting.

Duplicate dermal preparations were prepared for collagenase digestion by dialysis against 10 mM Tris, pH 6·8. The dialysed BMZ extract was incubated with 1 unit of purified bacterial collagenase in the presence of 1 μM calcium chloride for 30 min and then run on SDS-PAGE with a control non-collagenase-treated dermal extract.

Electrophoresis. Prior to SDS-PAGE, all dermal and epidermal samples were incubated in SDS sample buffer containing 10 mM Tris pH 6·8, 100 mM dithiothreitol and 4% SDS at room temperature for 30 min. Proteins were subjected to SDS-PAGE according to Laemmli[22] using a 7·5% gel. The resolved proteins were transferred on to nitrocellulose[23] and the resultant blots blocked overnight at 4°C in phosphate-buffered saline (PBS), pH 7·4 (0·15 M NaCl, 0·02 M NaPO4) containing 3% bovine serum albumin (BSA) and 0·02% sodium azide.

Table 1. Direct immunofluorescence. BMZ immunoreactants in perilesional and uninvolved tissue

Patient	Perilesional skin	Forearm skin	Oral mucosa	Conjunctival mucosa	Suction blister (forearm)
1	C3+IgG	C3+IgG	C3	C3+IgG	C3+IgG (roof)
2	C3	—	—	—	—
3	C3+IgG	—	C3+IgG	—	—

Antisera. Blotting patterns of the three LPP sera were compared to normal human sera, six BP sera, and to three LP sera on epidermal and dermal extracts and collagenase-digested dermal extracts. All sera were tested at dilutions of 1/5.

Probing. The nitrocellulose strips were probed with the primary antibody at dilutions of 1/5. The blots were then incubated with the appropriate peroxidase conjugated second antibody, rabbit anti-human IgG (Dako-Patts, Glostrup, Denmark). Antibody binding was detected by reacting the blots with PBS containing 0·5 mg/ml diaminobenzidine and 0·02% hydrogen peroxide.

HLA typing

This was performed using standard methods.

Results

Direct immunofluorescence

In all three cases there was a linear deposition of C3 along the BMZ in the perilesional skin. IgG was also present in two of the three cases. Direct IF was positive in one of three biopsies (IgG+C3) of uninvolved forearm, two of three biopsies of oral mucosa (one C3, the other IgG+C3), and one of three biopsies of conjunctival mucosa (IgG+C3) (Table 1). Only one suction blister was positive and showed epidermal binding of immunoreactors.

Table 2. Indirect immunofluorescence: results for IgG BMZ antibodies using different tissue substrates

	Human skin		Oral mucosa		Conjunctiva	Vagina	Pig's bladder	Placenta	
Sera	1 (ear)	2 (forearm)	1 (lip)	2				1	2
Patient 1	–	+	–	–	–	–	–	–	–
Patient 2	–	–	–	+	–	+	–	–	–
Patient 3	+	+	+	+	+	+	–	+	+
LP (three patients)	–	–	–	–	–	–	–	–	–
BP1	+	+	+	+	+	+	+	+	+
BP2	+	+	+	+	+	+	+	+	–
NHS	–	–	–	–	–	–	–	–	–

LP, lichen planus; BP, bullous pemphigoid; NHS, normal human serum.

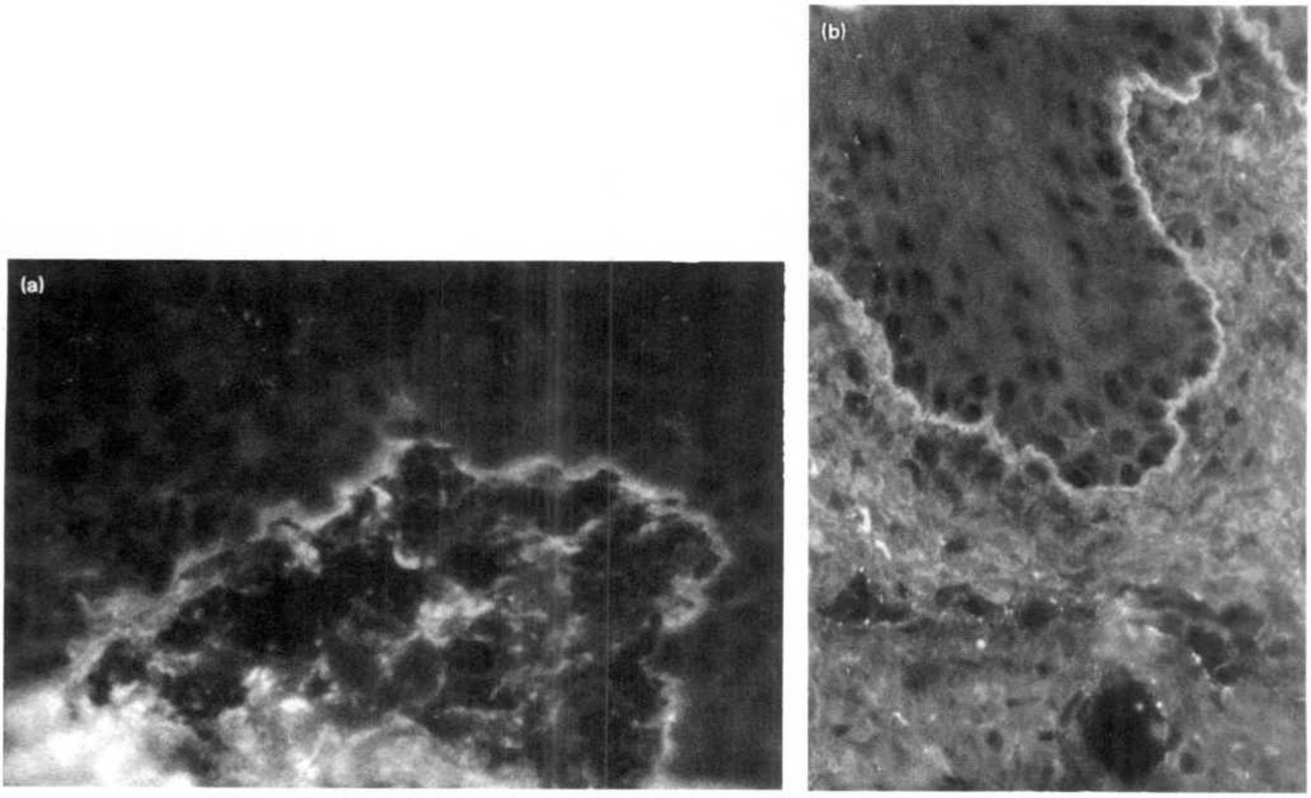

Figure 1. Indirect immunofluorescence showing IgG antibodies binding to BMZ of: (a) Conjunctival mucosa, (b) oral mucosa.

Indirect immunofluorescence

Two samples of human skin, oral mucosa and placenta each from different subjects were used as substrate (Table 2, Fig. 1). There was a lack of concordance for serum from one subject (Case 2) with regard to staining of oral mucosa, and from one subject (Case 1) for staining of skin. The IgG BMZ antigen was demonstrated in the skin, oral mucosa, conjunctival mucosa, vagina and placenta as was the BP antigen (Table 2).

Indirect IF showed circulating IgG antibody bound to the epidermal side of 1 M NaCl split skin and the epithelial aspects of split vagina (Table 3, Fig. 2), as did control BP antisera. There was no difference between samples split for differing periods (24–72 h) and at different temperatures (4/13°C).

None of the LPP patients' sera showed binding to pig bladder, which suggested that the antigen differed from BP. The control BP sera did bind to this tissue, confirming the results of Pothupitiya *et al.*[18]

Table 3. Indirect immunofluorescence comparing intact and 1 M NaCl split tissue

Sera	Skin	Split skin	Vaginal mucosa	Split vaginal mucosa
Patient 1	–	–	–	–
Patient 2	–	Epidermal	+	Epithelial
Patient 3	+	Epidermal	+	Epithelial
LP (three patients)	–	–	–	–
BP (two patients)	+	Epidermal	+	Epithelial
NHS	–	–	–	–

LP, lichen planus; BP, bullous pemphigoid; NHS, normal human serum.

Immunoelectron microscopy

Indirect immunoelectron microscopy revealed immune deposits in the lamina lucida in one out of three cases of LPP at a titre of 1/10 (Fig. 3). Direct immunoelectron microscopy also showed immunoreactants in the lamina lucida in two of three perilesional skin biopsies.

Immunoblotting

Sera from two LPP patients detected antigens in the epidermal extract only of 200 and 180 kDa (Fig. 4, lane 3). No binding was found in the dermal extract with or

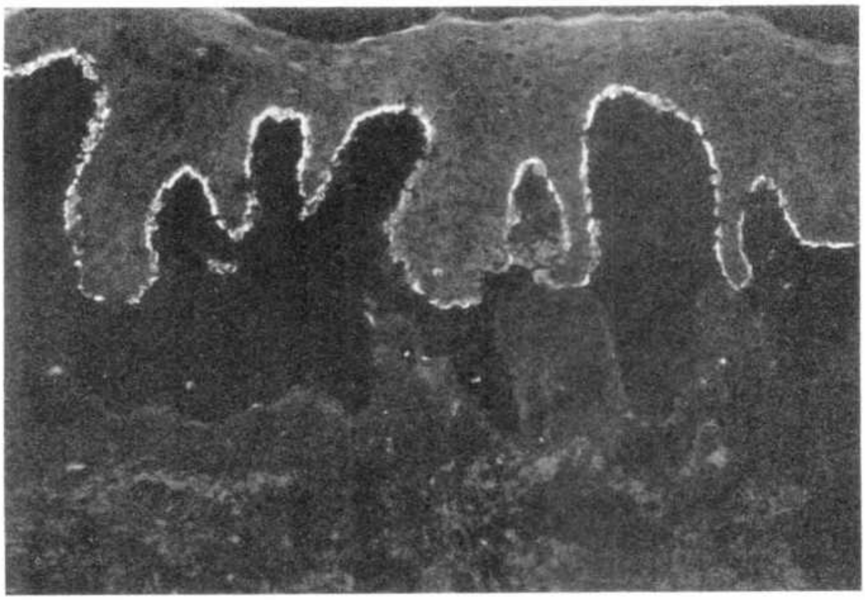

Figure 2. Indirect immunofluorescence microscopy of 1 M NaCl split skin showing IgG binding to the epidermal component.

without collagenase treatment. Serum from the third LPP patient failed to react at all but this was also negative by indirect immunofluorescence. Of the six BP control sera, five detected epidermal antigens of 220 kDa (Fig. 4, lanes 1 and 2) and one detected an epidermal antigen of 180 kDa (Table 4) in the epidermal extract only. No BP antisera reacted with the 200 kDa band in the epidermal extract. There was no reaction with the dermal extract. Control normal human serum and lichen planus serum (Fig. 4, lane 4) showed no reaction with either the dermal or epidermal extracts.

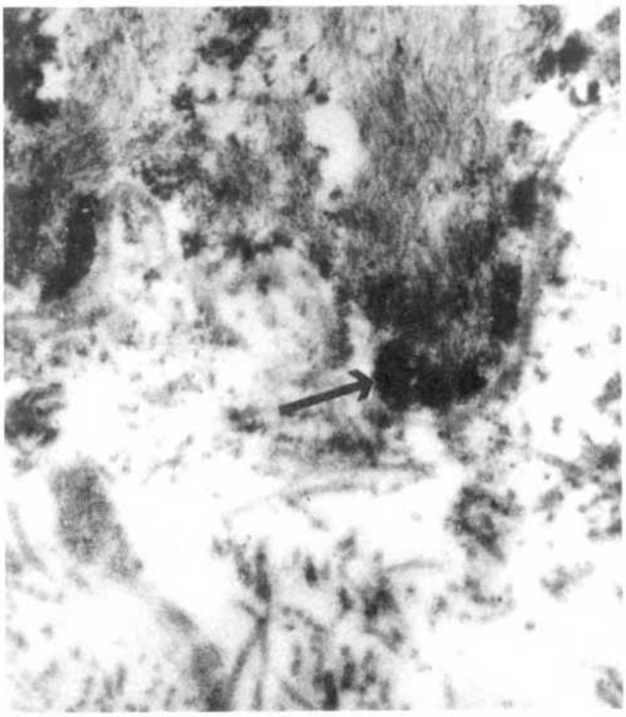

Figure 3. Indirect immunoelectron microscopy: immune deposits (arrows) in the lamina lucida.

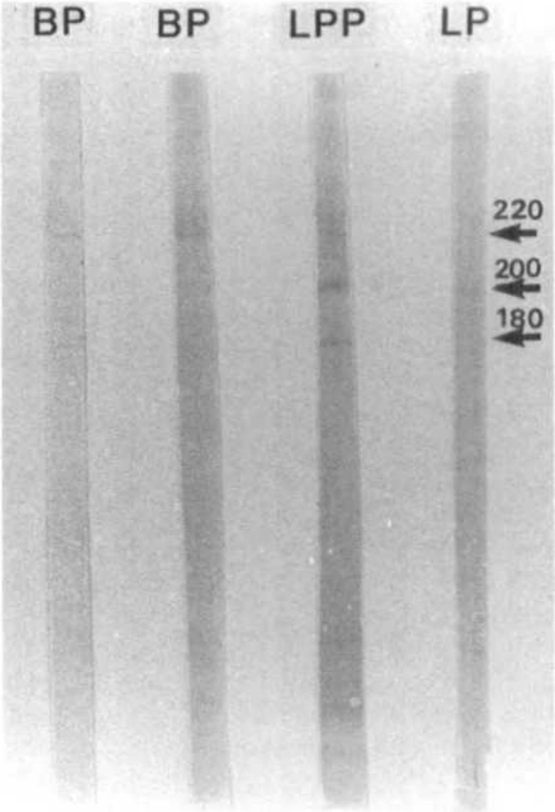

Figure 4. Immunoblotting: lanes 1 and 2 bullous pemphigoid sera, lane 3 lichen planus pemphigoides serum, lane 4 control lichen planus serum.

Table 4. Molecular weight (kDa) of antigens detected by immunoblotting of epidermal extract

LPP (Patient 1)	—	200	180
LPP (Patient 2)	—	200	180
LPP (Patient 3)	—	—	—
BP	220 (5)	—	180 (1)

HLA typing

The results show no trend (Table 5).

Discussion

The purpose of this study was to investigate the relationship between lichen planus pemphigoides and bullous pemphigoid and to determine whether the antigen in LPP is the classical BP antigen. Studies of the pathogenesis of classical lichen planus have emphasized the role played by cell-mediated immune responses, deposition of immunoreactant being secondary to lymphocyte-mediated epidermal cell damage.[24,25] In BP there is evidence that autoantibody binding to the BMZ in involved in the pathogenesis of the disease both *in vivo* and *in vitro*.[26,27] Thus the question arises as to whether the blistering found in LPP is due to binding of a specific antibody or to non-specific dermo-epidermal separation.

Previous immunofluorescence studies of bullous LP specimens have shown mixed results, some showing linear deposition of IgG at the BMZ[28,29] and others failing to confirm these findings. LPP is a rare disease, but we have had the opportunity to study three patients. Direct immunofluorescence showed linear deposition of C3 and in two cases IgG at the BMZ as in BP. Indirect immunofluorescence was positive on at least one substrate in all three cases. Multiple substrates were used as previous studies have demonstrated the expression of BP antigen in all stratified squamous epithelia including conjunctiva.[18,30] In our patients the distribution of the LPP antibody binding in stratified squamous epithelia by both direct and indirect immunofluorescence was identical to that of the BP antigen. On skin separated by suction blisters and on 1 M sodium-chloride split skin and vagina, both BP and LPP antigens were found at the epithelial border. Indirect and direct immunoelectron microscopy, when positive, confirmed that binding occurred within the lamina lucida, as in classical BP. Previous immunoelectron microscopic studies have shown immunoreactants in the lamina lucida.[9,14] These findings suggest that the LPP and BP antigens lie in the same position within the BMZ.

Table 5. HLA typing

Patient	A		B		C		DR	
1	11	29	35	14	4	8	1	(4)
2	3	28	8	14	8	(7)	(NT)	
3	3	—	51	65	8	—	1	—
	MT		DQ		BW			
1	(53)	—	(1)	(3)	—	6		
2	(NT)		(NT)		—	6		
3	—	—	1	—	4	(6)		

NT, not tested.

However, important differences were found. Firstly, the LPP sera did not react with transitional epithelium (pig's bladder) which has been shown to react with BP sera.[18] Secondly, protein blotting identified a distinct antigen. Classical BP antigen has been shown on immunoprecipitation and immunoblotting[31] to be a 230-kDa non-collagenous glycoprotein synthesized by epidermal keratinocytes, and this protein has now been partially cloned.[32] It appears to be a major hemidesmosome plaque protein[33–35] and two further specific components of the hemidesmosome of 180 kDa and 125 kDa have now been characterized together with plakoglobin.[36] Thus the reported heterogeneity of BP antigens as shown by immunoblotting is likely to reflect different molecular targets.[37–41] It is very difficult to compare results from different laboratories without reference sera or monoclonal antibodies, but we were able to use a well-characterized BP antiserum as an internal control. Immunoblotting sera from five patients with BP showed that four bound to the classical 230-kDa antigen identified by the reference serum. The single exception bound to the 180 kDa minor BP antigen. Two of the three LPP patients were positive on immunoblotting and both bound to an antigen at 200 kDa, clearly differing from those identified by the BP sera. This contrasts with the findings in a single patient with drug-induced lichen planus pemphigoides whose serum immunoprecipitated the classical BP antigen.[15]

Our series is too small to draw definitive conclusions, particularly with respect to HLA typing which is recorded for comparison with other later series. However, the suggestion that in LP, as in bullous lupus erythematosus (bullous LE), epidermal cell damage from liquefactive degeneration exposes basement membrane antigens to stimulate autoantibody production is appeal-ing. The BMZ is very complex, bearing many macromolecules, and multiple proteins are potential autoantibody targets following basal cell damage. In bullous LE, type VII collagen appears to be the target antigen,[42] whereas in a recent case of dermatomyositis pemphigoides (M.Glover, unpublished data) the antibodies bound to the classical BP antigen. It is of interest that sera from two of the patients bound to a unique determinant. Further molecular characterization is required to determine whether the LPP sera bind to the fusion proteins from the BP cDNA clones but this will clarify the exact nosological status of LPP.

References

1 Kaposi M. Lichen ruber pemphigoides. *Arch Derm Syph (Berlin)* 1892; **24**: 343–6.

2 Schreiner K. Lichen ruber pemphigoides und lichen ruber vesiculosus *Arch Dermatol Syph* 1930; **161**: 647–57.

3 Gawkrodger DJ, Stavropoulos PC, McLaren KM, Buxton PK. Bullous lichen planus and lichen planus pemphigoides–clinico-pathological comparisons. *Clin Exp Dermatol* 1989; **14**: 150–4.

4 Stingl G, Holubar K. Coexistence of lichen planus and bullous pemphigoid. An immunopathological study *Br J Dermatol* 1975; **93**: 313–20.

5 Rekant SI. Lichen planus and bullous pemphigoid. *Arch Dermatol* 1976; **112**: 1613.

6 Sobel S, Miller R, Shatin H. Lichen planus pemphigoides. Immunofluorescence findings *Arch Dermatol* 1976; **112**: 1280–3.

7 Saurat JH, Guinepain MT, Didierjean L *et al.* Coexistence d'un lichen plan et d'une pemphigoide bulleuse. Etude en immunofluorescence d'un 'lichen pemphigoide'. *Ann Dermatol Venereol* 1977, **104**: 368–74.

8 Morel P, Saurat J-H, Civatte J. Etude en immunofluorescence d'un nouveau cas de lichen plan bulleux *Ann Dermatol Venereol* 1978; **105**: 81–2.

9 Hintner H, Tappeiner G, Honigsmann H, Wolff K. Lichen planus and bullous pemphigoid. *Acta Derm Venerol* 1979; **59** (Suppl.): 71–6.

10 Collins NJ, Dowling JP. Lichen planus and bullous pemphigoid. *Aust J Derm* 1982; **23**: 9–13.

11 Mora RG, Nesbitt LT Jr, Brantley JB. Lichen planus pemphigoides. clinical and immunofluorescent findings in four cases. *J Am Acad Dermatol* 1983, **8**: 331–6.

12 Oomen C, Temmerman L, Kint A. Lichen planus pemphigoides. *Clin Exp Dermatol* 1986; **11**: 92–6.

13 Souteyrand P, Pierini AM, Fernandez Bussy R *et al* Lichen planus pemphigoides. Entity or association? *Dermatologica* 1981; **162**: 414–16.

14 Prost C, Tesserand F, Laroche L *et al.* Lichen planus pemphigoides. an immuno-electron microscope study. *Br J Dermatol* 1985; **113**: 31–6.

15 Okada N, Kitano Y, Miyagawa S *et al.* Expression of pemphigoid antigen by SV40-transformed human keratinocytes. *J Invest Dermatol* 1986; **86**: 399–401.

16 Lang PG, Maize JC. Coexisting lichen planus and bullous pemphigoid or lichen planus pemphigoides? *J Am Acad Dermatol* 1983; **9**: 133–40.

17 Venning VA, Frith PA, Bron A *et al.* Mucosal involvement in bullous and cicatricial pemphigoid: a clinical and immunopathological study. *Br J Dermatol* 1988; **118**: 7–15.

18 Pothupitiya GM, Wojnarowska F, Bhogal BS, Black MM. Distribution of the antigen in adult linear IgA disease and chronic bullous dermatosis of childhood suggests that it is a single and unique antigen. *Br J Dermatol* 1988; **118**: 175–82.

19 Kelly S, Wojnarowska F. The use of chemically split tissue in the detection of circulating anti-basement membrane zone antibodies in bullous pemphigoid and cicatricial pemphigoid. *Br J Dermatol* 1988; **118**: 31–40

20 Beutner EH, Nisengard RJ, Kumar V. Defined immunofluorescence: basic concepts and their application to clinical immunodermatology. In: *Immunopathology of the Skin* (Beutner EH, Chorzelski TP, Bean SF, eds). New York: John Wiley. 1979.

21 Prost C, Dubertret T, Fosse M *et al.* A routine immunoelectron-microscope technique for localising an antibody on epidermal basement membrane *Br J Dermatol* 1984; **110**: 1–7.

22 Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 1970, **227**: 680–5.

23 Towbin HT, Straehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc Natl Acad Sci USA* 1979; **76**: 4350–4

24 Bhan AK, Harrist TJ, Murphy GF, Mihm MC Jr. T cell subsets and Langerhans cells in lichen planus; *in situ* characterisation using monoclonal antibodies. *Br J Dermatol* 1981, **105**: 617–22.

25 Black MM..What is going on in lichen planus? *Clin Exp Dermatol* 1977, **2**: 303–10.

26 Gammon WR, Lewis DM, Carlo JR *et al.* Pemphigoid antibody mediated attachment of peripheral blood leukocytes at the dermal-epidermal junction of human skin. *J Invest Dermatol* 1980; **75**: 334–9.

27 Naito K, Morioka S, Ogawa H. The pathogenetic mechanisms of blister formation in bullous pemphigoid. *J Invest Dermatol* 1982; **79**: 303–6.

28 Baart De La Faille-Kuyper EH. Immunohistochemisch onderzoeck bij lichen ruber. *Ned Te Geneesijk* 1973; **117**: 473.

29 Baart de la Faille–Kuyper EH, Baart de la Faille H An immunofluorescence study of lichen planus *Br J Dermatol* 1974; **90**: 365–71.

30 Beutner EH, Chorzelski TS, Jordon RE *Autosensitization in Pemphigus and Bullous Pemphigoid III.* Springfield: Charles C. Thomas, 1970; 26–31.

31 Mueller S, Klaus-Kovtun V, Stanley JR A 230-kD basic protein is the major bullous pemphigoid antigen *J Invest Dermatol* 1989; **92**: 33–8.

32 Stanley JR, Tanaka T, Mueller S *et al.* Isolation of complementary DNA for bullous pemphigoid antigen by use of patients' autoantibodies. *J Clin Invest* 1988, **82**: 1864–70

33 Mutasim DF, Morrison LH, Takahashi Y *et al.* Definition of bullous pemphigoid antibody binding to intracellular and extracellular antigen associated with hemidesmosomes *J Invest Dermatol* 1989; **92**: 225–30.

34 Westgate GE, Weaver AC, Couchman JR. Bullous pemphigoid antigen localisation suggests an intracellular association with hemidesmomes. *J Invest Dermatol* 1985; **84**: 218–24.

35 Green KJ, Jones JCR. Interaction of intermediate filaments with the cell surface. In: *Intermediate Filaments.* (Goldman RD, Steinert PM, eds.) New York: Alan Arliss, 1989, 149–71.

36 Klatte DH, Kurpakus MA, Grelling KA, Jones JCR. Immunochemical characterisation of three components of the hemidesmosome and their expression in cultured epithelial cells. *J Cell Biology* 1989; **109**: 3377–90.

37 Labib RS, Anhalt GJ, Patel HP *et al.* Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 1986, **136**: 1231–5

38 Cook AL, Hanahoe THP, Mallet RB, Pye RJ. Recognition of two distinct major antigens by bullous pemphigoid sera. *Br J Dermatol* 1990; **122**: 435–44.

39 Zhu X-J, Bystryn J-C. Heterogeneity of pemphigoid antigens. *J Invest Dermatol* 1983; **80**: 16–20.

40 Reano A, Thivolet J, Staquet MJ, Fargier MC. Utilisation de la technique d'immunotransfert pour l'etude des anticorps presents dans le serum de malades atteints de pemphigoid bulleuse. *Ann Dermatol Venereol* 1988; **115**: 11–18.

41 Bernard P, Didierjean L, Denis F *et al.* Heterogeneous bullous pemphigoid antibodies: detection and characterization by immunoblotting when absent by indirect immunofluorescence *J Invest Dermatol* 1989; **92**: 171–4.

42 Gammon WR, Woodley DT, Dole KC, Briggaman RA. Evidence that the anti-basement membrane antibodies in bullous eruption of systemic erythematosus recognise epidermolysis bullosa autoantigen. *J Invest Dermatol* 1985; **84**: 472–6.

British Journal of Dermatology (1991) 125, 272–278. ADONIS 000709639100215E

Long-term survival and preservation of natural killer cell activity in a xeroderma pigmentosum patient with spontaneous regression and multiple deposits of malignant melanoma

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Accepted for publication 27 March 1991

Summary A 67-year-old man with xeroderma pigmentosum (XP) originally presented with malignant melanoma at the age of 28 years. This recurred 22 years later and subsequently numerous primary and secondary melanomas developed on the skin, several of which underwent spontaneous regression. Despite a marked lymphopenia, the proportion of natural killer cells was elevated and it is proposed that this led to the regression of the melanomas. Skin-derived fibroblasts from the patient were more sensitive to UVC ($D_{10} \sim 3$ J/m^{-2}) than those from normal individuals ($D_{10} \sim 15$ J/m^{-2}). The fibroblast culture was shown to be defective in excision repair with $<10\%$ of residual activity compared with controls. No assignment to a complementation group has yet been made. There was an elevated frequency of mutants resistant to 6-thioguanine in the circulating T lymphocytes.

The sun-sensitive, cancer-prone autosomal recessive syndrome xeroderma pigmentosum (XP) has been the subject of many studies at the clinical, cellular and molecular levels. In a review of 830 cases Kraemer *et al.*[1] showed that patients had a dramatically increased incidence of both melanoma and non-melanoma skin cancer, and that sunlight was involved in the development of these tumours. At the cellular level XP can be divided into two groups, those defective in excision repair of UV-induced DNA damage and those competent for excision repair. The former group comprises seven complementation groups which have been assigned by cell-fusion studies. The second excision-proficient group (XP variants) comprises a single set and cells are defective in post-replication (daughter strand) repair.

Survival studies with fibroblasts in culture reveal that the excision-defective cells are hypersensitive to the lethal action of UVC and those chemicals which produce bulky adducts in DNA.[2,3] The XP variant cells are only marginally more sensitive than controls to UVC although this sensitivity can be enhanced by the inclusion of caffeine in the post-irradiation medium.[4] Both classes of XP cells have been shown to be hypermutable by UVC to 6-thioguanine (6-TG) resistance.[5,6] In addition, the circulating T lymphocytes of XP patients show an elevated frequency of 6-TG resistance in comparison with normal control lymphocytes.[7] Hypermutability and hypersensitivity in this disease can thus be related to cancer through defects in DNA repair. The validity of this association has been questioned as a result of observations made in Cockayne's syndrome (CS) and in particular trichothiodystrophy (TTD), in which hypermutability and defects in repair have also been recognized but no increased incidence of cancer has been recorded.[8] In an attempt to reconcile these observations Lehmann and Norris[9] proposed a two-gene hypothesis based on the observation that XP patients, unlike those with CS and TTD, appeared to be defective in natural killer (NK) cell function.

We have studied the clinical manifestations, immunology and cellular responses in a XP patient who presented with a history of self-healing melanotic lesions. In our patient, despite a marked lymphopenia, NK cell activity and numbers were preserved.

Methods

Case report

A 28-year-old man presented in 1950 with a lesion on

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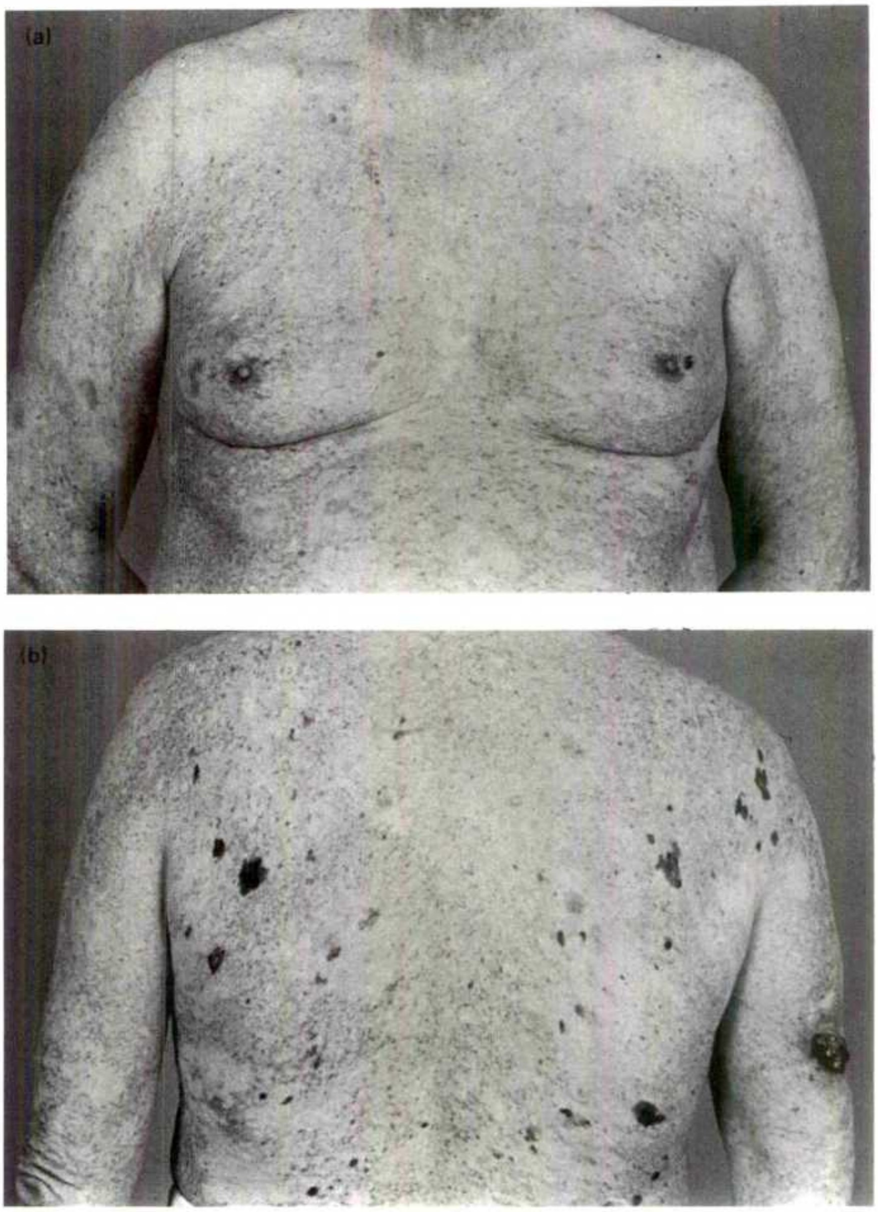

Figure 1. Patient's (a) chest and (b) back in November 1988 showing areas of hypopigmented skin corresponding to sites of melanomas which have resolved spontaneously. Numerous melanomas are present in addition to a fungating polypoid melanoma on the right upper arm.

his forehead which had the clinical appearance of a pyogenic granuloma. This was removed and the histology was reported as an infected cellular naevus. He was first seen by a dermatologist in 1962 and was noted to have marked freckling, telangiectasia and atrophic patches of skin in the sun-exposed areas, suggesting a possible diagnosis of xeroderma pigmentosum.

In 1972 he presented with a skin lesion on his neck which was diagnosed clinically as a sebaceous cyst. The histology was reported at the time as a metastasis of an anaplastic carcinoma but detailed investigation failed to identify a primary lesion. In 1976 he developed pigmented lesions on the face, anterior chest and back. Two lesions were biopsied and were diagnosed histologically as malignant melanoma, one specimen showing features suggestive of a primary lesion. The patient refused treatment and for the next 12 years attended intermittently for excision of particularly large or troublesome pigmented lesions. During this time he remained healthy despite the numerous melanomas. Examination of the individual melanomas revealed a wide diversity. Their appearance ranged from typical dark and evenly pigmented lesions of 2–3 cm diameter to non-pigmented cutaneous nodules and pedunculated, ulcerated pigmented lesions. These were distributed over face, trunk, upper and lower limbs and many of them remitted

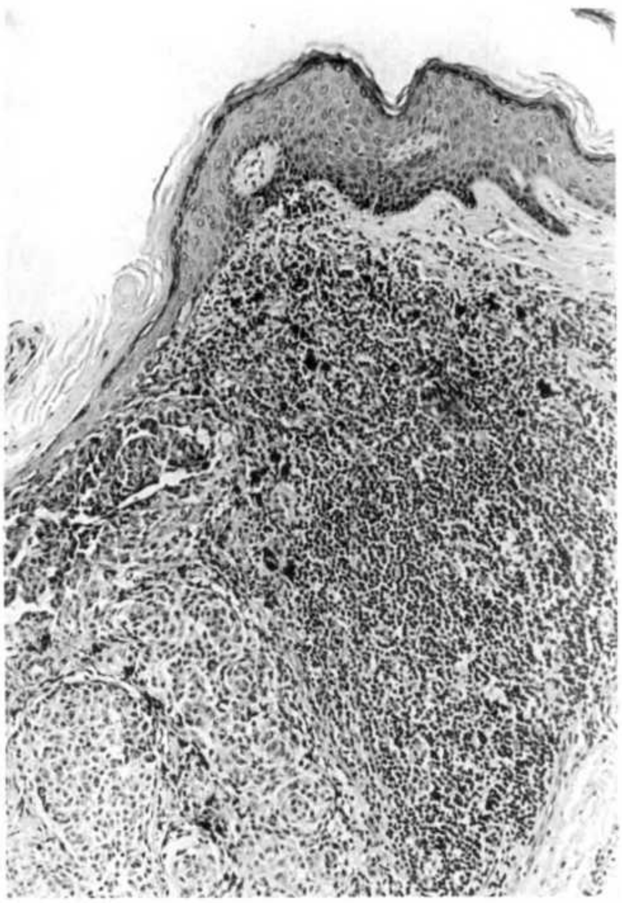

Figure 2. Primary malignant melanoma excised from the upper back in November 1988 (Haemotoxylin and eosin, x132).

spontaneously. In 1985 he was clinically free from melanoma. Since then he has developed a number of further melanomas (Fig. 1), of which some showed the histological features of primary lesions (Fig. 2) whilst others had the histological appearance of secondary lesions.

Apart from his many and varied melanotic skin lesions he also had numerous freckles, telangiectasia and atrophic areas with large hypopigmented patches corresponding to sites of melanoma which remitted spontaneously. The sun-exposed skin had features of severe actinic damage, despite the absence of an outdoor occupation and avoidance of excess sun exposure since childhood. There was no family history of xeroderma pigmentosum, dysplastic naevus syndrome or malignant melanoma.

In 1988 he developed haemoptysis which settled without treatment. Investigations to determine the cause of this included chest X-ray, bronchoscopy, CT scan of thorax and sputum cytology, all of which were normal. He remains in good health but with numerous cutaneous deposits of malignant melanoma.

Histology

A review of the histology of the original biopsy from 1950 showed it to be a secondary deposit of malignant melanoma and the 1972 biopsy from the neck showed a similar appearance. There were five melanomas excised in 1982 of which two were primary lesions and three were secondary lesions. A biopsy from 1984 showed a secondary lesion and three biopsies from 1988 all showed primary melanomas. Typical lesions consisted of clumps and ribbons of spindle-shaped or polygonal malignant cells with a variable amount of melanin pigment in the cell cytoplasm as confirmed by special stains. Considerable nuclear atypia and frequent mitoses were present in some of the specimens. Primary lesions showed melanoma cells in continuity with the basal layer of the epidermis (Fig. 2). Secondary cutaneous lesions showed an overlying epidermis with a normal basal layer separated from the melanoma by a thin layer of normal dermis.

Assessment of immune function

Lymphocyte subset analysis by flow cytometric determination of cell surface markers (FACScan and Simulset Reagents, Becton Dickinson) showed a very abnormal profile of leucocytes (Table 1). The percentages of $CD3^+$ cells and $CD8^+$ cells were well below the normal range while that of $CD4^+$ cells was normal. However, the absolute numbers of all these cells were low as a result of a marked lymphopenia. Further investigation to assess T-cell function was refused by the patient. The proportion of circulating NK cells as defined by the Leu IIC (CD16) and Leu 19 markers was markedly elevated at 47% (normal range 6–23%) resulting in normal absolute numbers of cells in spite of the severe lymphopenia. Direct NK cytotoxicity measured against the human erythromyeloid leukaemia cell line K562 by means of a 4-h ^{51}Cr-release assay[10] was normal (specific cytotoxicity 45% with an effector-cell:target-cell ratio of 50:1) compared with cytotoxicity in normal controls (46 + 18 <SD>).

Cellular studies

Fibroblast survival. The responses to the lethal effects of UVC, using standard procedures[11] of fibroblast cultures from the patient (which we have designated XP6BR),

Table 1. Flow cytometric analysis of lymphocyte subsets

Cell marker	Patient		Normal ranges	
	%	Abs.No.($\times 10^6$/ml)	%	Abs.No($\times 10^6$/ml) ($\pm$1SD, $n=22$)
CD3 lymphocytes (total T)	36	0·29	59–79	1·01–2·39
CD4 lymphocytes (T helper)	35	0·28	33–48	0·58–1·44
CD8 lymphocytes (T suppressor)	17	0·14	28–38	0·50–1·12
T-helper/suppressor ratio	2·06		0·93–1·58	
Activated T cells	3	0·02	1–6	0–0·17
B cells (DR+ve, non-T cells)	9	0·07	8–15	0·17–0·37
NK cells	47	0·38	6–23	0·14–0·52
MHC unrestricted CTL	1	0·01	0–4	0–0·12

Total number lymphocytes per ml: 0·8 million.

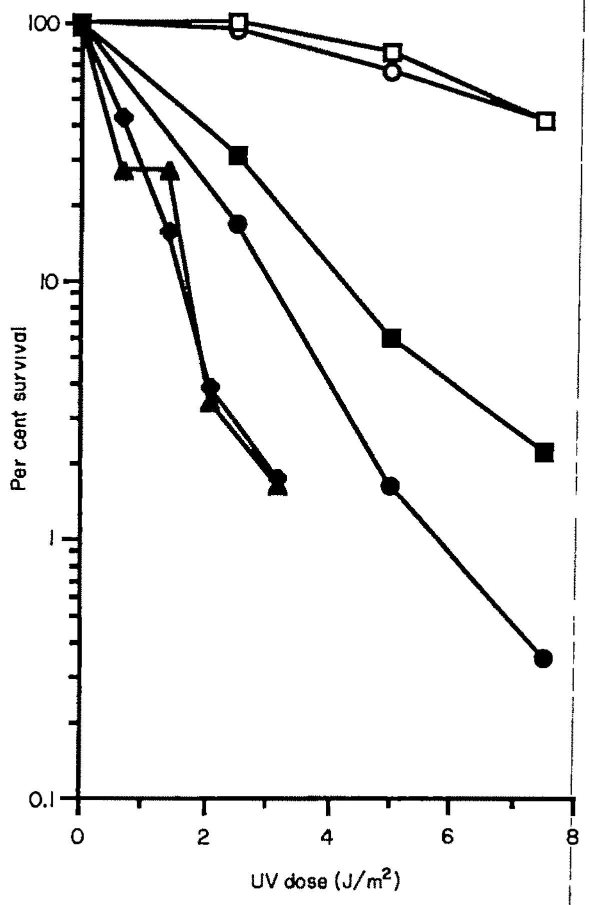

Figure 3. Cell survival in fibroblasts from normal and UV-sensitive individuals. Colony forming ability of cells following irradiation with different fluences of UVC. GM730 (–□–) and 1BR3 (–○–) are normal cell strains, CS4BR (–■–) is Cockayne's syndrome; XP1BR (–◆–) and XP3BR (–▲–) are xeroderma pigmentosum and XP6BR (–●–) is the patient.

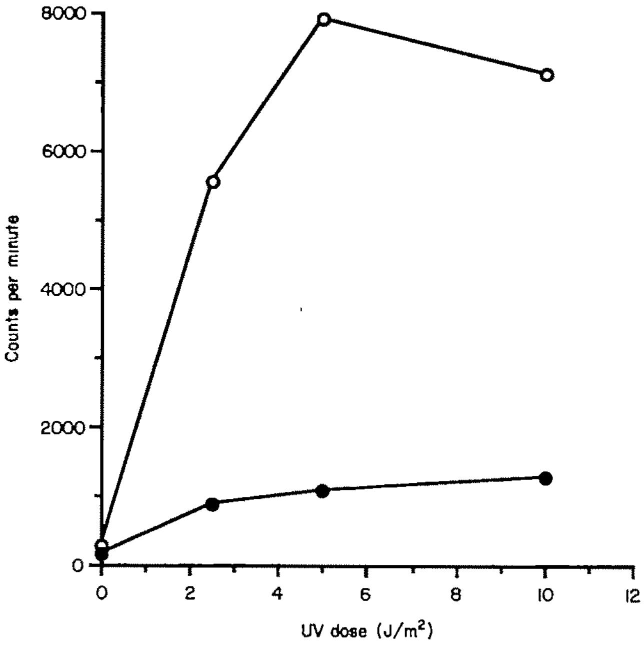

Figure 4. Unscheduled DNA synthesis in fibroblasts from a normal individual (1BR3, –○–) and the patient (XP6BR, –●–)

two controls and representative XP and CS cell strains which were included for scaling purposes are shown in Figure 3. XP6BR cells were unambiguously more sensitive than controls, although not as sensitive as the other XP cultures.

Unscheduled DNA synthesis. The results of a single set of observations with fibroblast cultures using standard procedures[12] are shown in Figure 4 and reveal the

Table 2. Mutant frequency to 6-thioguanine resistance in circulating T lymphocytes in XP6BR and normal donors from two experiments

Donor	Age	Status	Smoking	Experiment number	CE%†	Mutant frequency per clonable cell × 10^{-6}
XP6BR	67	XP	No	1a*	38	29·6
				1b	29	49·3
				2	53	29·1
64	51	Normal female	Yes	1a	54	15·1
				1b	54	13·3
42	55	Normal male	No	2	41	10·0
2	49	Normal female	Yes	2	56	18·8
34	26	Normal male	Yes	2	44	14·2

* Experiment 1 was split between two different serum batches, indicated by a and b

† CE%, per cent cloning efficiency of the T lymphocytes in the plates without selective agent.

The mean (unweighted) mutant frequency for XP6BR = $35{\cdot}01 \times 10^{-6}$. When compared to all normal non-smokers in the 40–70 age group, this represents a 5·43 fold increase ($t=5{\cdot}21$, 80d.f., $p<0{\cdot}001$). Our current results for this age group show that smokers have a 2·09 fold increase over non-smokers ($t=5{\cdot}12$, $p<0{\cdot}001$).

extensive defect in excision repair (<10% of residual activity) in XP6BR.

Mutation to 6-thioguanine resistance. Our methodology for measuring the frequency of 6-TG-resistant T lymphocytes in circulating blood was described in detail elsewhere.[13] Briefly, cells in suspension after the overnight culture period were counted and cloned in 96-well microtitre^{R} trays in the presence 6-TG at 2×10^4 cells per well to determine the mutant fraction, and at three cells per well in non-selective medium to determine the plating efficiency of the T cells. The mutant frequency to 6-TG resistance from two independent blood samples for XP6BR is shown in Table 2. In the first, the available control sample was from a smoker; in the second, two smokers and one non-smoker were used. A comparison with both smoking and non-smoking controls is important because we have demonstrated a significant effect of smoking habit on 6-TG-resistant mutant frequency.[13] Our patient who is a non-smoker had an elevated mutant frequency.

Discussion

Our cellular studies confirmed the clinical diagnosis of XP by showing hypersensitivity to the lethal action of UVC on fibroblasts.[2,3] There was also an unambiguous defect in excision repair[14] although an assignment to a complementation group has not yet been made. The mutant frequency in the circulating T lymphocytes of XP6BR was elevated in common with all other XP patients investigated by us to date[7] and this might be regarded as a potential diagnostic characteristic at the cellular level. The origin of such mutants is open to speculation. Fibroblasts[6] and lymphoblastoids[15] from XP patients are hypermutable and thus the elevated mutant frequency in blood-derived cells might be due to exposure of lymphocytes to UV as they pass through the skin of the subject.

The clinical status of our patient is of considerable interest because of the self-healing and recurrent nature of his melanomas. Using established histological[16,17] and clinical[18] criteria the prognosis for this patient appeared to be very poor, yet he has remained well for nearly 40 years with cutaneous metastatic melanoma. A similiar case has been reported of a patient with xeroderma pigmentosum who developed multiple melanomas with cerebral metastases which resolved spontaneously, the patient remaining in good health 25 years later.[19] Our patient developed a secondary deposit of malignant melanoma 22 years after excision of the original lesion, having been apparently disease-free in the interim period. Late recurrence of cutaneous malignant melanoma has been regarded as local, regional or distant metastases occurring 10 or more disease-free years after initial diagnoses.[20] Fifty-seven cases of this rare and poorly understood phenomenon have been described previously.[21]

Spontaneous regression of malignant melanoma is well recognized and is defined as the partial or complete disappearance of melanoma in the absence of any treatment or in the presence of therapy considered inadequate to significantly alter the natural course of the malignancy.[22] Spontaneous regression of metastic melanoma is less common than that of primary melanoma and has a prevalence of approximately 0·25%.[23] Of 30 reported cases of spontaneous regression of malignant melanoma,[22-24] nine followed incomplete surgical excision or biopsy and in eight no factors contributing to regression were evident. Associated factors identified in the remaining 13 cases were potential immune stimulation (five cases), infection (two cases), altered endocrine status (four cases) and inadequate radiotherapy (two cases). One patient survived for 20 years, one for 12 years, two for 11 years and the remainder for 10 years or less. Features known to be associated with spontaneous regression were exhibited by many of the melanomas in our patient including lesions with a Clark level of III or less, a diameter greater than 10 mm, areas of whiteness, an association with solar elastasis, and an anatomical location other than the head or neck.[25]

The mechanism underlying spontaneous regression of malignant melanoma is unclear, but may be due to destruction of tumour cells by immune effector mechanisms. Immune surveillance processes comprise adaptive T-cell mediated responses directed against tumour-associated antigens and cytotoxicity by NK cells which do not require sensitization and may be important as protection against weakly immunogenic tumour cells such as in melanoma.[26,27] Defects in both adaptive cell-mediated immunity and NK-cell activity have been described previously in xeroderma pigmentosum.[28-30] Bridges first proposed than an immune defect may be crucial to development of malignancies in xeroderma pigmentosum[31] and Lehmann and Norris have subsequently suggested that this might be inherited independently of the DNA repair abnormality.[9] An inherited defect in NK activity has also been described in familial melanoma.[26]

In this patient there was a marked reduction in the total T-cell population, but the number of cells positive for the NK markers did not fall, and thus the proportion of cells with NK function was increased. In spite of a severe lymphopenia, the patient had both normal NK cell absolute numbers and activity. These results suggest that NK cells form an expanded population in our patient and suggest a potential role for NK cells in the immune surveillance of melanoma.

Acknowledgments

We thank Professor Rona Mackie, Department of Dermatology, University of Glasgow for reviewing the histology and Dr M.H.L.Green for statistical support. S.A.Harcourt, A.Waugh, D. Beare and B.C.Broughton from MRC Cell Mutation Unit contributed to the cellular studies. We also thank the doctors who have contributed to the care of this patient, in particular Mr J.Grogono, Mr B.Neal and Mr W.Fountain. Professor B.A.Bridges provided many helpful comments. The work was supported, in part, by E.C.Grants B16 042 UK (H) and EV4V-0037 UK.

Note added in proof

This individual's cells are now assigned to complementation group C (A.Joose and W.Vermeulen, personal communication).

References

1 Kraemer KH, Lee MM, Scotto J. Xeroderma pigmentosum, cutaneous ocular and neurologic abnormalities in 830 published cases. *Arch Dermatol* 1987; **123**: 241–50.

2 Maher VM, McCormick JJ, Grover P *et al.* Effect of DNA repair on the cytotoxicity and mutagenicity of polycyclic hydrocarbon derivatives in normal and xeroderma pigmentosum fibroblasts. *Mutation Res* 1977; **43**: 117–138.

3 Lehmann AR, Kirk-Bell S, Arlett CF *et al.* Repair of ultraviolet light damage in a variety of human fibroblast cell strains. *Cancer Res* 1977; **37**: 904–10.

4 Arlett CF, Harcourt SA, Broughton BC. The influence of caffeine on cell survival in excision-proficient and excision-deficient xeroderma pigmentosum and normal cell strains following ultraviolet irradiation. *Mutation Res* 1976; **33**: 341–6.

5 Maher VM, Ouellette LM, Curren RD *et al.* Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal cells. *Nature* 1976; **261**: 593–5.

6 Arlett CF, Harcourt SA. Variation in response to mutagens amongst normal and repair-defective human cells. In: *Induced Mutagenesis, Molecular Mechanisms and their Implications for Environmental Protection* (Lawrence CW, ed.). New York: Plenum Press, 1982; 249–66.

7 Tates AD, Bernini LF, Natarajan AT *et al.* Detection of somatic mutants in man: HPRT mutants in lymphocytes and hemoglobin mutants in erythrocytes. *Mutation Res* 1989; **213**: 73–82.

8 Lehmann AR. Cockayne's syndrome and trichothiodystrophy: defective repair without cancer. *Cancer Reviews* 1987; **7**: 82–103.

9 Lehmann AR, Norris PG. DNA repair and cancer: Speculations based on studies with xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *Carcinogenesis* 1989; **10**: 1353–56.

10 Ortaldo JR, Bonnard GD, Herberman RB. Cytotoxic recovery of human lymphocytes cultured *in vitro*. *J Immunol* 1977; **119**: 1351–7.

11 Cole J, Arlett CF. The detection of gene mutations in cultured mammalian cells. In: *Mutagenicity Testing—A Practical Approach* (Venitt S, Parry JM, eds). Oxford: IRL Press, 1984; 233–73.

12 Lehmann AR, Stevens S. A rapid procedure for measurement of DNA repair in human fibroblasts and for complementation analysis of xeroderma pigmentosum cells. *Mutation Res* 1980; **69:** 177–90.

13 Cole J, Green MHL, James SE *et al.* Human population monitoring: a further assessment of factors influencing measurements of thioguanine-resistant mutant frequency in circulating T-lymphocytes. *Mutation Res* 1988; **204:** 493–507.

14 Cleaver JE. Defective repair replication of DNA in xeroderma pigmentosum. *Nature* 1968; **218:** 652–6.

15 Tatsumi K, Toyoda M, Hashimoto T *et al.* Differential hypersensitivity of xeroderma pigmentosum lymphoblastoid cell lines to ultraviolet light mutagenesis. *Carcinogenesis* 1987; **8:** 53–7.

16 Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Annals Surgery* 1972; **172:** 902–8.

17 Clark WH Jr, From L, Bernardino EA *et al.* The histogenesis and biologic behaviour of primary human malignant melanomas of the skin. *Cancer Res* 1969; **29:** 705–27.

18 MacKie RM, Carfrae DC, Cochran AJ. Assessment of prognosis in patients with malignant melanoma. *Lancet* **ii:** 1972; 455–6.

19 Ronchese F. Spontaneous bleaching of melanotic freckles. *Arch Dermatol* 1966; **94:** 739–41.

20 Raderman D, Giler S, Rothem A, Ben-Bassat M *et al.* Late metastases (beyond ten years) of cutaneous malignant melanoma. *J Am Acad Dermatol* 1986; **15:** 374–8.

21 Briggs JC, Ibrahim NBN Late recurrence of cutaneous melanoma. *J Am Acad Dermatol* 1988; **18:** 147–9.

22 Nathanson L. Spontaneous regression of malignant melanoma. A review of the literature on incidence, clinical features and possible mechanisms. *Natl Cancer Inst Monogr* 1976; **44:** 67–76.

23 Bodurtha AJ. Spontaneous regression of malignant melanoma. In: *Human Malignant Melanoma* (Clark WH, Goldman LI, Mastrangelo JM, eds). New York: Grune and Stratton, 1979; 227–41.

24 Mikhail GR, Gorsulowsky DC. Spontaneous regression of metastatic malignant melanoma. *J Dermatol Surg Oncol* 1986; **5:** 497–500.

25 Trau H, Kopf AW, Rigel DS *et al.* Regression in malignant melanoma. *J Am Acad Dermatol* 1983; **8:** 363–8.

26 Bystryn J-C Immunosurveillance and melanoma. *J Invest Dermatol* 1989; 92 (Suppl): 318–20.

27 Hersey P, Edwards A, Honeyman M, McCarthy WH *et al.* Low natural killer cell activity in familial melanoma patients and their relatives. *Br J Cancer* 1979; **40:** 113–22.

28 Wysenbeek AJ, Weiss H, Duczyminer-Kahana M *et al.* Immunologic alterations in xeroderma pigmentosum patients *Cancer* 1986, **58:** 219–21

29 Norris PG, Limb GA, Hamblin AS *et al.* Immune function, mutant frequency and cancer risk in the DNA repair defective genodermatoses xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *J Invest Dermatol* 1990; **94:** 94–100.

30 Gaspari AA, Fleisher TA, Kraemer KH. Reduced natural killer cell activation and diminished induction of interferon-γ activity in leukocytes from patients with xeroderma pigmentosum. *J Invest Dermatol* 1990; **94:** 527.

31 Bridges BA. How important are somatic mutations and immune control in skin cancer? Reflections on xeroderma pigmentosum. *Carcinogenesis* 1981; **2:** 471–2.

British Journal of Dermatology (1991) **125**, 279–287. ADONIS 000709639100216R

SOCIETY PROCEEDINGS

Proceedings of the 255th Meeting of the Netherlands Society for Dermatology and Venereology, Utrecht, 2 February 1991

PRESIDENT: PROFESSOR H.A.M.NEUMANN
HOST: PROFESSOR W.A.VAN VLOTEN

Received 22 April 1991

Case Reports

Rothmund–Thomson syndrome

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History. A 12-year-old girl had skin lesions on the cheeks, arms, hands, buttocks and feet, since early life. The affected sites showed erythema, telangiectasia and subcutaneous atrophy. She had dystrophic nails, normal scalp hair and sparse eyebrows.

She was of normal intelligence and health. She had recurrent infections and chilblains of hands and feet during the winter. Family history was negative for skin diseases.

Examination. Her height was normal for her age. She had a high-pitched voice and there was frontal bossing with a saddle deformity of her nose, and she had defective dentition (Fig. 1). Ophthalmological examination showed no abnormalities.

Laboratory tests. Extensive investigation for humoral or cellular defects did not reveal any abnormality. Radiological examination showed sclerosis of the epiphyseal lines.

Histology. The epidermis was normal, but there were strikingly dilated blood vessels in the papillary dermis.

Discussion. The Rothmund–Thomson syndrome is a rare syndrome inherited as an autosomal recessive and with developmental defects.[1,2] In several cases the syndrome is associated with ectodermal dysplasia. Characteristic clinical features are early onset of poikiloderma, sparseness of the eyebrows, familial juvenile cataracts, small

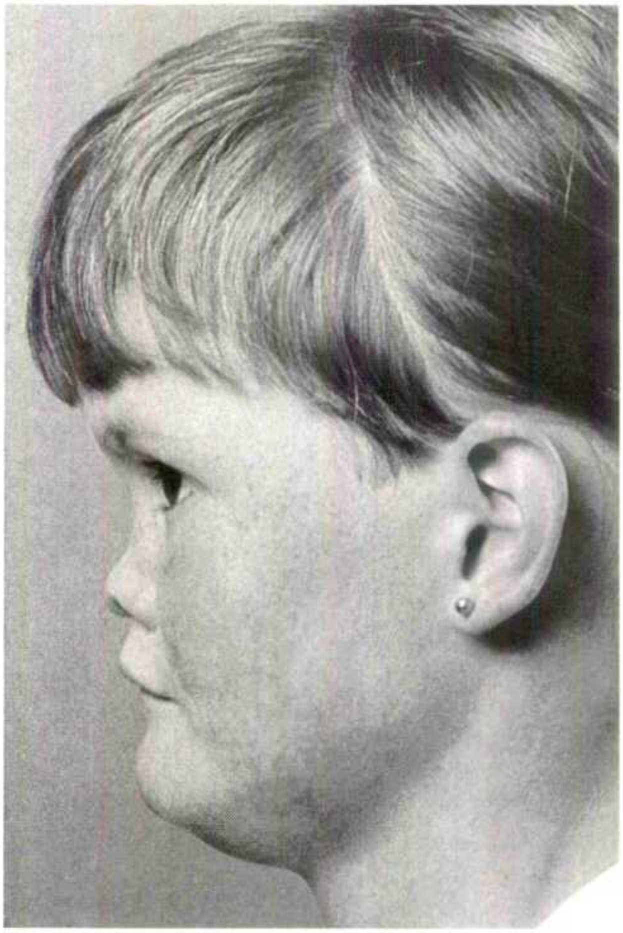

Figure 1. Rothmund–Thomson syndrome.

hands and bone defects, sensitivity to sunlight, hypogonadism, dystrophic nails, defective dentition, hyperkeratosis, and mental retardation.[3]

References

1 Pagon RA. Rothmund–Thomson Syndrome. In: Gomez MR. *Neurocutaneous Diseases* (Gomez MR, ed). Boston: Butterworths, 1987: 136–42.

2 Rook A, Davis R, Stevanovic D. Poikiloderma congenitale Rothmund–Thomson syndrome. *Acta Derm Venereol (Stockh)* 1959; **39**: 392–420.

3 Berg E, Chuang TY, Cripps D. Rothmund–Thomson syndrome, a case report, phototesting, and literature review. *J Am Acad Dermatol* 1987; **17**: 332–8.

Type I allergy to latex

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History. A woman aged 28 years developed urticarial swellings 1 year ago after using ordinary rubber gloves at home and swelling of her lips while blowing up rubber balloons. Generalized urticaria developed on using condoms. She had had atopic eczema for many years.

Laboratory and other tests. The patient was an atopic as judged by positive skin tests to common inhalant allergens. Scratch epicutaneous tests with pieces of wet rubber and skin prick tests with natural latex were strongly positive after 20 minutes. Epicutaneous testing (delayed-type reaction) with rubber chemicals was negative.

A high (4+) level of specific IgE antibodies for latex was found on the RAST test (Central Laboratory Red Cross Blood Transfusion Service, Amsterdam).

Discussion. Since Nutter[1] first reported IgE-mediated contact urticaria to rubber there have been several other reports showing that rhinitis, asthma and even anaphylaxis can also occur in association with exposure to rubber.[2] The allergen is probably a water-soluble protein of natural latex. The frequent use of rubber condoms in relation to preventive measures against AIDS may lead to an increased prevalence of sensitization to latex. If patients allergic to latex require medical or dental treatment or surgery, the dentist, or surgeon should use plastic gloves or gloves of Elastyren (styrene–butadiene polymer), to prevent anaphylactic reactions. Predisposing factors are atopic constitution and hand dermatitis.[3]

References

1 Nutter AF. Contact urticaria to rubber. *Br J Dermatol* 1979; **101**: 597–8.

2 Frosch PJ, Wahl R, Bahmer FA, Maasch HJ. Contact urticaria to rubber gloves is IgE-mediated. *Contact Dermatitis* 1986; **14**: 241–5.

3 Turjanmaa K. Incidence of immediate allergy to latex gloves in hospital personnel. *Contact Dermatitis* 1987; **17**: 270–5.

Incontinentia pigmenti (Bloch–Sulzberger)

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History. A 9-month-old female patient presented with lesions on the legs that had started at the age of 4 months with pruritus and vesicles and which were linearly distributed. There was no consanguinity of the parents and no congenital defects in their families. The mother had not had an abortion and the development of the child was normal.

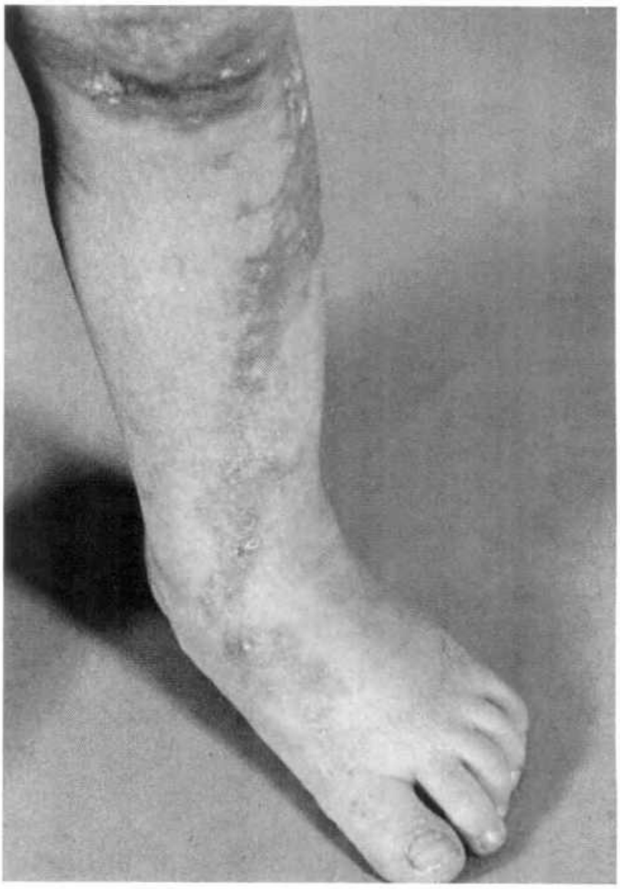

Figure 1. Incontinentia pigmenti.

Examination. There were linear, grey/brown areas of hyperpigmentation on both legs (Fig. 1). Some portions were elevated. Similar lesions were present on the right arm, the axilla and in the groin. There were no neurological, eye or developmental defects.

Laboratory tests. There was no eosinophilia in the blood at the age of 1 year.

Histology. A skin biopsy showed hyperkeratosis, papillomatosis and there was acanthosis of the epidermis. There was intraepidermal keratinization with some dyskeratotic cells. In the dermis there was a cellular infiltrate with some eosinophils and incontinence of pigment with some melanophages.

Discussion. This is a rare neurocutaneous genodermatosis described for the first time in 1926 by Bloch and in 1928 by Sulzberger.[1]

There is X-linked inheritance, without racial preference and the disease has a lethal course in boys with spontaneous abortion.[2] Cutaneous signs (and hairs and nails) as well as neurological defects (spastic paralysis, microcephaly, mental subnormality), developmental defects (dental abnormalities, dwarfism) and eye defects (optical atrophy, nystagmus) are reported in many cases.[3] The skin lesions run through three phases: vesicular, verrucous and a final phase where there are linear and whorled areas of hyperpigmentation that can later resolve.

References

1 El Benhawi MO, George WM. Incontinentia pigmenti. *Cutis* 1988; **41**: 259–62.

2 Carney RG. Incontinetia pigmenti. A world statistical analysis. *Arch Dermatol* 1976; **112**: 535–42.

3 Gordon H, Gordon W. Incontinenti pigmenti: clinical and genetical studies of two familial cases. *Dermatologica* 1970; **140**: 150–68.

Relapsing polychondritis

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History. A 62-year-old man suffered for 5 years with a painful lesion on the left auricle. Extensive purulent exudation was present, but repeated bacterial cultures did not reveal any pathological micro-organisms. There was no improvement with oral tetracyclines.

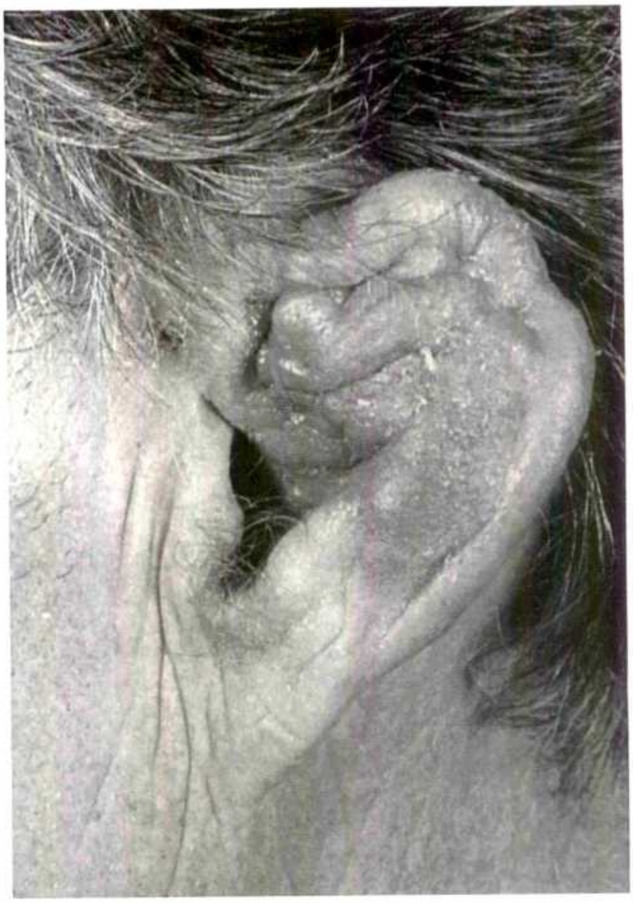

Figure 1. Relapsing polychondritis. Characteristically the earlobe is not involved.

Examination. There was erythema and induration of a large part of the left auricle with deformity of the superior part. A fistula was present with a seropurulent exudate. The earlobe was unaffected (Fig. 1).

Laboratory tests. There was a moderately increased number of leucocytes: $10{\cdot}0–17{\cdot}7 \times 10^6$/ml. The ESR was 12–24 mm/h.

Histology. A skin biopsy showed a leucocytoclastic vasculitis with nuclear dust and a mixed infiltrate with eosinophils. No cartilage was present.

Treatment. The patient was treated with prednisone 30 mg daily resulting in a full remission in weeks. A relapse occurred after slowly reducing the dose to 7·5 mg daily but on doubling the dose of prednisone the patient improved.

Discussion. The diagnosis was based on the clinical features and was supported by the histological and laboratory findings.[1] The condition should, however, be differentiated from erysipelas.

Reference

1 McAdam LPM, O'Hanlan MA, Bleustone R, Pearser CM. Relapsing polychondritis. Prospective study of 23 patients and a review of the literature. *Medicine* 1976; **55:** 193–215.

Unexplained myalgia in a patient with linear IgA disease

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History. A 24-year-old woman developed at the age of 17 an itchy blistering dermatoses on the trunk and extremities. She had a myalgia of the proximal parts of the limbs and felt tired. She was diagnosed as having linear IgA dermatoses and was treated with dapsone, but this was stopped because of haemolysis. An exacerbation of her skin condition then occurred with enormous blisters, itching, myalgia and general malaise.

Examination. There were erythematous, vesicular/bullous and erosive lesions on the trunk and extremities, especially the palms of the hands, and soles of the feet, varying from several mm to several cm. The individual lesions had annular and polycyclic forms (Fig. 1).

Laboratory tests. Radiodiagnostic examination of the intestinal tract showed a normal pattern of the jejunum and there was no villous atrophy on the jejunal biopsy.

There was a slightly elevated serum IgA.

Skin–muscle–fascia biopsy (histology, histochemistry and direct immunofluorescence) of the dermis showed a perivascular mononuclear infiltrate. On electromyography, there were abnormalities as seen in myositis.

Histology. The skin biopsy showed a subepidermal bulla with an infiltrate of neutrophils and eosinophils. At the tops of the dermal papillae there were neutrophilic microabscesses. In the upper part of the dermis there was a perivascular lympho-monocyte infiltrate and to a lesser extent there were neutrophils and eosinophils.

Direct immunofluorescence of lesional and normal skin showed a band-like deposition of IgA along the basement membrane zone in a linear and partly granular pattern.

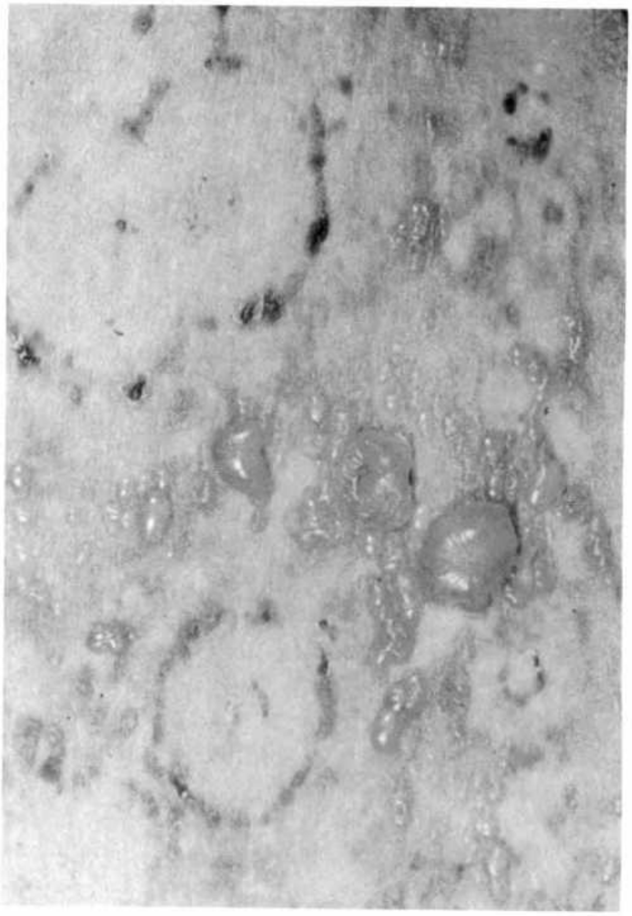

Figure 1. Annular bullous lesion in linear IgA disease.

Treatment. Dapsone in a dosage of 100 mg daily controlled the disease, but caused haemolysis and was then stopped. The condition was then controlled with sulphamethoxypyridazine 500 mg daily.

Discussion. We cannot explain the myalgia in our patient with linear IgA disease. It is still uncertain as to whether this disease is part of the spectrum of disorders that include dermatitis herpetiformis or parapemphigus.[1,2]

Often there is deposition of IgA, IgM and IgG in the skin in Sjögren's disease and in dermatomyositis, however in our case the ANF was negative and is usually positive in these diseases. Also in our patient the sedimentation rate was normal and this makes the diagnosis of polymyalgia rheumatica unlikely. The myalgia in our patient responded well to therapy with sulphamethoxypyridazine.

References

1 Wojnarowska F. Linear IgA disease of adults. In: *Management of*

Blistering Diseases (Wojnarowska F, Bruggeman RA, eds). London: Chapman and Hall Medical, 1990; 105–19.

2 Leonard JN, Haffenden GP. Linear IgA disease in adults. *Br J Dermatol* 1982; **107**: 301–16.

Treatment of pyoderma gangrenosum with cyclosporin

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History. Following trauma a 69-year-old man developed a painless ulcer on the right lower leg which failed to heal. After some time identical lesions spontaneously developed on the right calf and on the flexor side of the left upper leg. He was known to have a seronegative rheumatoid arthritis and Hashimoto's hypothyroidism. There were no signs of bowel disease.

Examination. In the pretibial region and on the calf of the right lower leg, and on the flexor side of the left upper leg, there were three ulcers with a diameter of 10–20 cm, red purple in colour and with pus covering their centres and with ragged and undermined violaceous borders.

Laboratory tests. Autoantibodies against thyroid tissue could be detected. Cultures from swabs of the ulcer were negative for mycobacterial and fungal organisms.

Histology. In the deep dermis there was ulceration with an acute inflammatory infiltrate. At the margins of the ulcer a more chronic inflammatory infiltration was present.

Treatment. The patient was treated with cyclosporin A 5 mg/kg/day with checks of the blood pressure, renal function and the blood level of cyclosporin. An improvement was seen after 1 week of cyclosporin A treatment. After 3 months the ulcers healed and after 6 months' therapy the dose of cyclosporin A was slowly reduced. However, recurrence of the lesions made us resume therapy.

Discussion. Seventy to eighty per cent of the patients with pyoderma gangrenosum have an associated systemic disease.[1] Our patient suffered from seronegative rheumatoid arthritis and Hashimoto's disease.

A wide variety of therapeutic modalities have been used for the condition and current oral therapies include prednisone, sulphonamides, clofazimine, minocycline and immunomodulators such as azathioprine and cyclophosphamide. Recent reports of the use of cyclosporin A in the treatment of pyoderma gangrenosum are encouraging,[2,3] but the mode of action is as yet unclear, probably there is a suppression of lymphokine production by activated T cells.

References

1 Schwaergerle SM, Bergfeld WF, Senitzer D, Tidrich RT. Pyoderma gangrenosum: a review. *J Am Acad Dermatol* 1988; **18**: 559–68.

2 Curley RK, MacFarlane AW, Vickers CFH. Pyoderma gangrenosum treated with cyclosporin A. *Br J Dermatol* 1985; **113**: 601–4.

3 Magid ML, Gold MH. Treatment of recalcitrant pyoderma gangrenosum with cyclosporin. *J Am Acad Dermatol* 1989; **20**: 293–4.

Generalized essential telangiectasia

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History. A 50-year-old woman developed 2 years ago purple red pin-sized lesions grouped on the lower legs. There was then rapid progression of the lesions over the rest of the body.

She only had a cosmetic problem and no symptoms suggestive of systemic disease.

Examination. On the trunk and limbs and especially on the lower legs there were extensive sheets of small telangiectatic vessels apart from the palms, soles, the face and under the bra.

Laboratory tests. The liver and kidney function tests were all normal and 5 hydroxy indole acetic acid was not detected in the urine. The ANF was negative.

Histology. There were telangiectatic vessels in the upper dermis.

Treatment. None.

Discussion. The cause of generalized essential telangiectasia is still unknown. The usual age of onset is in middle life and almost exclusively in women. The primary lesions are pin-sized red purple vascular macules appearing in groups and can involve any area of the body and especially the lower limbs.[1]

The lesions do not usually bleed. A striking feature in our patient was the absence of lesions in the submammary area. Hypostasis seems to play an important part in causing the telangiectasia and external compression with bandages may diminish the progression of the disease.

References

1 McGrae JO, Winkelman RK. Generalized essential telangiectasia. *JAMA* 1963; **21**: 909–13.

Carbon dioxide laser treatment of multiple tricho-epitheliomas

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History. A 35-year-old woman had multiple flesh-coloured papules, located in the nasolabial folds and on the upper lip from the age of 13. Since that time the lesions have gradually enlarged, and new ones have appeared on the scalp, forehead, chin and upper part of the trunk.

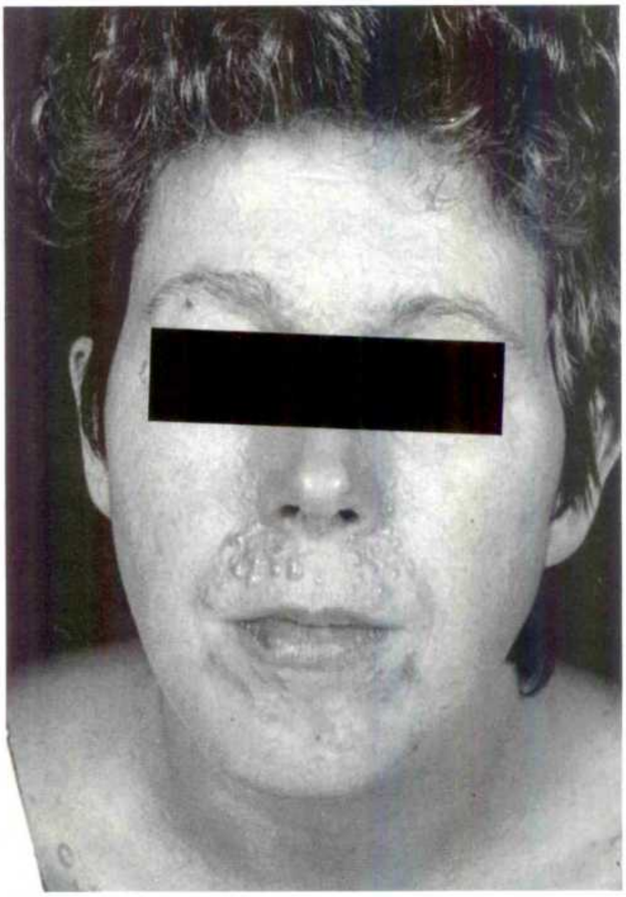
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Figure 1. Multiple tricho-epitheliomas before treatment.

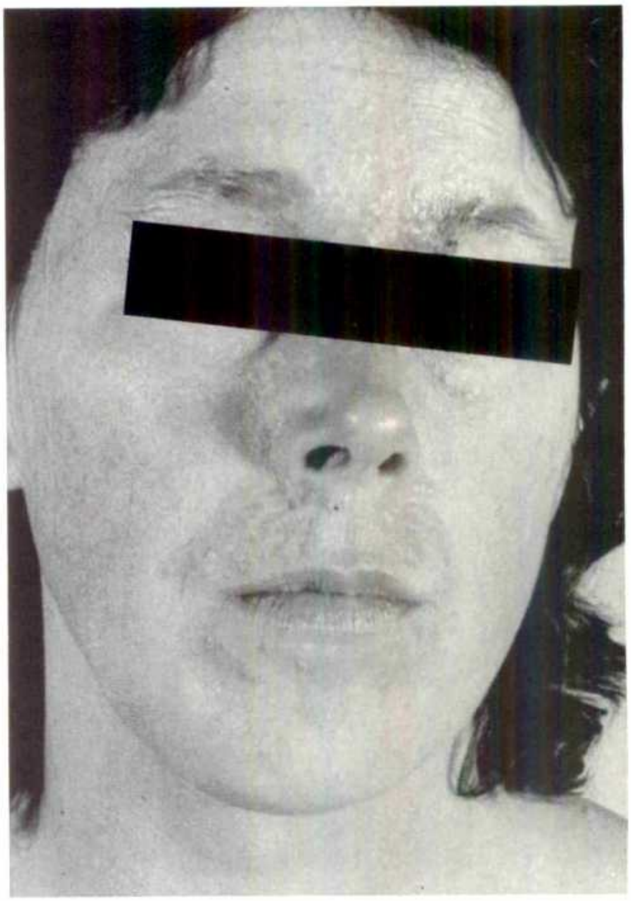
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Figure 2. Multiple tricho-epitheliomas after treatment with carbon dioxide laser.

Her mother, a brother and a sister are known to have similar skin tumours.

Examination. On the scalp, on the face, especially on the forehead, in the nasolabial folds and on the upper part of the trunk, multiple pink coloured firm papules and nodules were found ranging from 1 to 10 mm in diameter.

Histology. Underneath a normal epidermis there was a well circumscribed tumour composed of several small keratinous cysts surrounded by interconnecting sheets and strands of basaloid epithelial cells with peripheral palisading. The tumour cells were embedded in a collagen stroma, with a sparse lymphohistiocytic cellular infiltrate.

Treatment. The patient (Fig. 1) was treated with a carbon dioxide laser and prior to therapy local anaesthesia of 2% xylocaine was used. The lesions were treated in five separate sessions applying 4–5 W (3–40 W/cm^2) each time. No specific post-operative treatment was given. The cosmetic result was very satisfactory with only minimal scarring (Fig. 2).

Discussion. The condition of multiple tricho-epithelioma is rare and is inherited as an autosomal dominant.[1] The facial lesions cause considerable cosmetic disability. Different kinds of therapy have been described for the treatment of tricho-epitheliomas, including surgical excision, cryotherapy, dermabrasion, electro-cautery curettage, topical 5-fluorouracil, vitamin A and X-ray therapy. The results have been inconsistent and recurrence of the lesions has been frequently reported. Patients with multiple tricho-epitheliomas have been successfully treated with the carbon dioxide laser[2,3] and this is what happened with our patient. At the present time, 9 months after treatment there has been no recurrence of the treated lesions.

References

1 Gray HR, Helwig EB. Epithelioma adenoides cysticum and solitary tricho-epithelioma. *Arch Dermatol* 1963; **87:** 102–14.

2 Wheeland RG, Bailin PL, Kronberg E. Carbon dioxide (CO_2) laser vaporization for the treatment of multiple tricho-epithelioma. *J Dermatol Surg Oncol* 1984; **10:** 470–5.

3 Sawchuk WS, Heald PW. CO_2 laser treatment of trichoepithelioma with focused and defocused beam. *J Dermatol Surg Oncol* 1984; **10:** 905–7.

Infantile acne

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History. A 10-month-old boy had severe infantile acne with many papules, pustules and comedones from the age of 3 weeks. He was otherwise healthy. There was no improvement with local and systemic treatment with erythromycin.

Examination. A healthy boy without evidence of an endocrinopathy with papules, pustules and comedones on the forehead, cheeks and chin (Fig. 1). The rest of the skin was normal.

Treatment. Local therapy with 5% salicylic acid, 3% resorcinol in a cream during the day and 0·05% vitamin A acid cream at night produced a marked improvement within several weeks.

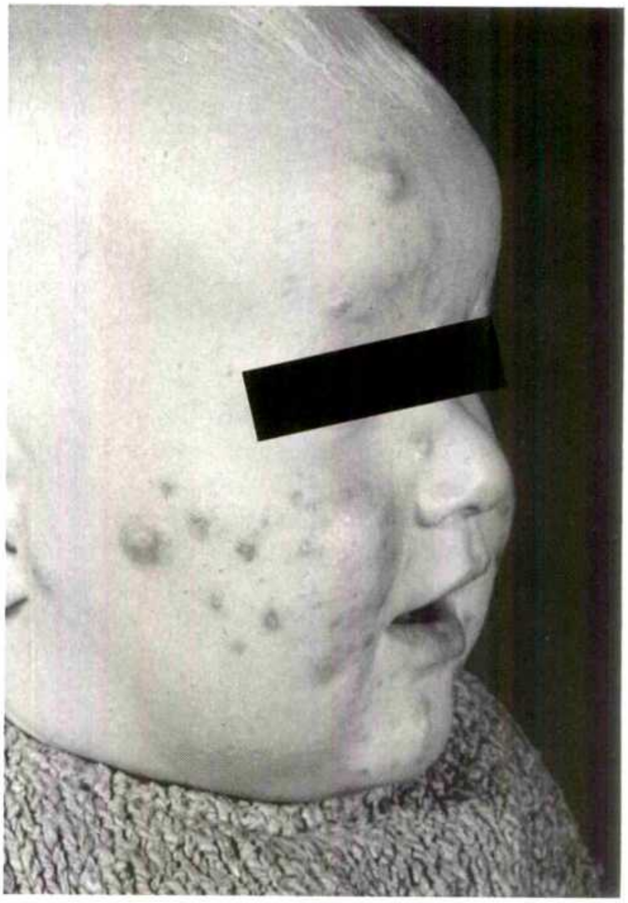
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Figure 1. Infantile acne.

Discussion. Infantile acne is a relatively rare disease, seen more often in boys than in girls. The lesions are usually located on the face, but sometimes occur on the back and chest. The pathogenesis of infantile acne is not fully understood.[1] Most cases require no treatment, but if the condition is severe then therapy with keratolytics is advised.[1] Patients with infantile acne may later develop severe acne as a teenager.[2]

References

1. Ebling FJG, Cunliffe WJ. The sebacous glands. In: *Textbook of Dermatology* (Rook *et al.*, eds.). Oxford: Blackwell Scientific Publications, 1986; 1897–1936.

2. Hellier FF. Acneiform eruptions in infancy. *Br J Dermatol* 1954; **66:** 25–30.

Merkel cell tumour

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History. A woman aged 79 years developed in May 1990 a nodule on the forehead. Histological examination was suggestive for a basal cell carcinoma. A few months later she was seen again, this time with two nodules in the same area and the tumours were removed by plastic surgery. In August two firm nodules appeared, one adjacent to the left eyebrow and the other on the nose.

Histology. In the deep dermis there was a solitary tumour that consisted of closely spaced cells with round vesicular nuclei and scanty ill-defined, granular cytoplasm (Fig. 1). Mitotic figures were present. Immunoperoxidase studies were positive for neuron-specific enolase and the diagnosis was that of a local recurrence of a Merkel cell tumour.

Treatment. The lesions were treated with radiotherapy and during the 6 months of follow-up there have been no further recurrences.

Discussion. A Merkel cell tumour is a rare and aggressive form of skin cancer.[1-3] It is thought to arise from the cells of the APUD system (amine precursor uptake decarboxylation).[3] The lesions appear as raised blue nodules which may arise on any body site. It is mostly seen on the face of elderly people. The differential diagnosis includes: lymphoma, squamous cell carcinoma, malignant melanoma and metastases of an oat-cell carcinoma of the bronchus. The natural history of this tumour is characterized by local recurrence in 30% of cases, regional lymph node metastases in 65% and distant metastases in 40%.[3] This case illustrates the need to perform a wide excision with a margin of 3–5 cm. In the case of a large primary lesion or metastasis, radiotherapy or chemotherapy should be considered.[1,2]

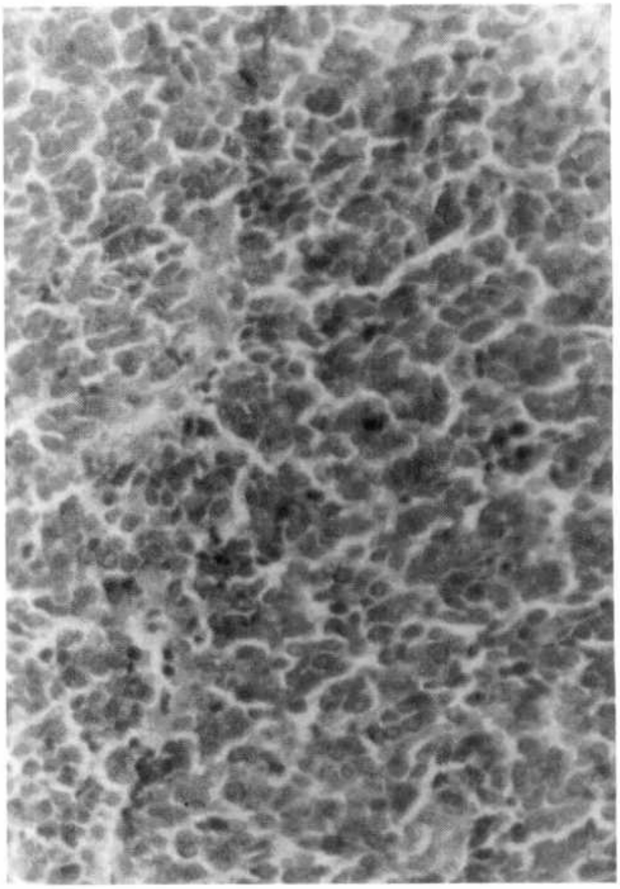

Figure 1. Merkel cell tumour. Closely spaced cells with round vesicular nuclei and scanty cytoplasm (× 100).

References

1 Hanke CW, Conner AC, Temofeew R, Lingeman R. Merkel cell carcinoma. *Arch Dermatol* 1989; **125:** 1096–100.
2 Wynne CJ, Kearsley JH. Merkel cell tumour, a chemosensitive skin cancer. *Cancer* 1988; **62:** 28–31.
3 Warner T, Uno H, Reza Hafez G *et al.* Merkel cells and Merkel cell tumours. *Cancer* 1983; **52:** 238–45.

Summaries of Papers

Botany and dermatology

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Contact with plants and flowers may cause a variety of dermatoses: allergic contact dermatitis, toxic reactions and phytophotodermatitis. Recently we performed a retrospective analysis regarding patch testing with fresh cut flowers in patients with hand eczema. *Alstroemeria* (Peruvian Lily) and *Chrysanthemum* most frequently caused a delayed-type hypersensitivity—6/16 ($\approx$38%) and 5/11 ($\approx$46%), respectively, and most of these patients had a professional contact with flowers: floral shop worker or horticulturist.[1,2] It did not matter which part of the cut flower was used for patch testing. The flower, leaf or stem all gave the same results. No correlation was found with positive patch tests with components of the European standard tray like fragrance mix, colophony or balsam of Peru. In some patients the forearms and face were affected as well as the hands. In the kitchen some vegetables (onion, garlic, chicory and celery), citrus fruits and spices (nutmeg, cloves, vanilla and laurel) are all notorious allergens.

Plants can also have a direct noxious effect on the skin: urticaria by nettles (*Urtica*), dermatitis by proteolytic enzymes from pineapple, by calcium oxalate crystals from narcissus/hyacinth bulbs or by phorbol esters from Euphorbiaceae.[3] Among the indigenous plants in The Netherlands, giant hogweed (*Heracleum sphondylium*) produces the most cases of phytophotodermatitis. In the vegetable garden parsley and parsnip are incidental causes of this photodermatosis. Nowadays the psoralen-rich cut flower *Ammi majus*, imported from Africa, is fashionable, but up to now no cases of phytophotodermatitis have been reported.

References

1 Ketel WG van, Verspijck Mijnssen GAW, Neering H. Contact eczema from alstroemeria. *Contact Dermatitis* 1975; **1**: 323–4.
2 Thiboutot DM, Hamory BH, Marks JJG. Dermatoses among floral shop workers. *J Am Acad Dermatol* 1990; **22**: 54–8.
3 Hausen BM. Allergie pflanzen-Pflanzen allergene. *Handbuch und Atlas der Allergologie-induziererenden Wild- und Kulturpflanzen*. Landsberg/München: Ecomed Verlagsgesellschaft, 1988.

Bath-PUVA for the treatment of psoriasis

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The objective was to study the effectiveness of bath PUVA in the treatment of patients with psoriasis vulgaris. An open pilot study was carried out at the psoriasis day care centre. Twenty patients (nine women, 11 men; mean age 42 years) with moderate plaque psoriasis were randomly selected. The minimal phototoxic dose (MPD) of each patient was determined on the arm after bathing for 10 min in an aqueous solution containing 0·5 or 1 mg/l of 8-methoxypsoralen (8-MOP). The patients were then treated with UVA after a bath of 10 min in the 8-MOP solution. The concentration of 8-MOP was 0·5 or 1 mg/l.

At each treatment the time of UVA irradiation was increased by 20% up to a maximum of 20 min and the treatment was carried out three times a week. To record the absorption of 8-MOP during the bath therapy, the serum concentration was measured using high-pressure liquid chromatography.

The results were evaluated using the PASI score for each patient at the beginning and end of treatment. After an average of 31·7 treatments, the PASI had dropped from 18·1 to 1·5, an improvement of 91·7%. In all patients the treatment was well tolerated. In all the patients treated the serum level of 8-MOP could not be detected (threshold 10 mg/ml).

Bath PUVA appears to be an effective for the treatment of patients with psoriasis,[1,2] although it is not suited for lesions in the scalp. Less side-effects are to be expected as compared to regular oral PUVA treatment.

References

1 Fischer T. Studies on UV light treatment of psoriasis. *PhD Thesis*, University of Upsala, 1977.
2 Vaatainen N, Hannuksela M, Karvonen J. Longterm local briocalen photochemotherapy in psoriasis. *Dermatologica* 1981; 163: 229–31.

British Journal of Dermatology (1991) **125**, 288–290. ADONIS 0007096391002176

Comment

White cells, skin blood flow and venous ulcers

About 100,000 people in the United Kingdom suffer from venous ulceration of the leg,[1] a prevalence of 0·15%. It is generally agreed that the underlying haemodynamic abnormalities are venous reflux and raised venous pressure, and particularly, an inability to reduce this pressure on exercising the limb.[2,3] How this persistently raised venous pressure is translated into skin ulceration remains a matter of speculation. Abnormal arteriovenous shunts, leading to reduced nutritive skin blood flow, were postulated in the 1940s and 1950s[4–6] but their significance was disproven by Partsch's group using microsphere tracing.[7] In 1982, Browse and Burnand demonstrated the existence of pericapillary fibrin cuffs which they suggested acted as a diffusion barrier to the passage of oxygen and other nutrients from the capillary bed to the skin surface.[8] This led to a model of ulcer pathogenesis based on skin hypoxia—a model that was generally accepted until very recently. However, the ineffectiveness of fibrinolytic treatment[9,10] has led to a questioning of the relative importance of fibrin in the mechanism of venous skin damage. In addition there are theoretical reasons for doubting whether fibrin actually impedes the diffusion of oxygen through tissue. It has been pointed out that the water content of biological fibrin is so large as not to limit diffusion of a small molecule like oxygen.[11] We examined the clearance of 133-xenon, a molecule four times bigger than oxygen which theoretically should diffuse half as quickly. No difference in the clearance rate of this isotope could be demonstrated from liposclerotic skin when compared with normal skin.[12]

Schmid-Schonbein *et al.*[13] and, more recently, Lawrence *et al.*[14] have demonstrated that reduction of the pressure gradient across the capillary bed from the arterial to the venous system leads to increased white cell margination in the post-capillary venule, probably as a consequence of decreased shear stress between these white cells and the endothelium. The overall effect is to enhance white cell–endothelial contact and adhesion. In 1987, Moyses *et al.* found that in normal subjects, white blood cells became sequestered in the leg when venous pressure was elevated by placing the limb in a dependent position.[15] The degree of this sequestration was shown to be greater in patients with deep venous insufficiency.[16] We subsequently confirmed the phenomenon of white-cell sequestration during raised venous pressure in a study of normal forearms, in which 15 min of tourniquet inflation to a pressure of 80 mmHg led to a rise in the haematocrit of 18% but a fall in the white cell/red cell ratio of 11%, indicating selective retention of white cells in the hand.[17]

This led to the formulation of a hypothesis proposing that skin ulceration in venous disease is the result of inappropriate white-cell activation caused by abnormally increased contact with venular endothelium, with consequent release of proteolytic enzymes and free radicals which produce the tissue damage.[18] We have investigated this possibility in two ways: by examining the sequestration of white cells in venous hypertension both functionally and histologically and by examining the effect on microcirculatory function of manoeuvres known to result in such sequestration.

Capillary microscopy, using the technique described by Fagrell,[19] was used in an initial study.[20] Patients were examined in the supine position after sitting for 30 min, then after a period of lying for 30 min and finally after sitting for a further period of 30 min. The number of visible capillary loops per unit area was determined in patients with normal limbs, varicose veins and with lipodermatosclerosis (LDS). Only in those patients with LDS were there significant alterations in the capillary counts with fewer capillary loops. The number of loops increased when the leg was elevated and fell when it was placed in a dependent position. We suspected that this was a consequence of white cell trapping.

Skin biopsies were then taken from 19 subjects, divided into three groups: five with uncomplicated varicose veins, six with LDS, and eight with LDS and a healed ulcer. Punch biopsies under local anaesthesia were taken from the supramalleolar skin while the patients lay supine. An observer, blinded to the diagnosis, counted the numbers of white cells in the superficial 0·5 mm of skin, in a total of 2880 slides. Subjects with normal skin had a mean of 6·2 white cells per mm^2; those with LDS and no previous ulcer had a mean of 45 and those with LDS and a history of venous ulceration had a mean of 217. These were highly significant differences.[21] The studies indicate that white cells are more numerous in liposclerotic skin than in normal skin but do not prove that they have a causative role. Skin damage from whatever cause is likely to result in an inflammatory response. Any experiment designed to show their mere presence, or even their presence in an activated form, cannot by its nature prove that they are the initiating factor responsible for skin damage. The pattern of falling capillary counts with rising venous pressure is, however, what would be expected were capillary occlusion occurring in these circumstances.

Knowing that raised venous pressure leads to leucocyte sequestration in the leg, we studied the effect of the same rise in pressure on microcirculatory function in normal skin. If a perfusion deficit caused by white cell/endothelial interaction is the pathogenetic mechanism underlying venous skin damage, it would be predicted that a fall in microcirculatory efficiency would occur over a time span similar to that over which white cells become sequestered—that is, in less than 30 min. We used laser-Doppler velocimetry to assess microcirculatory function in 17 normal limbs; the specific parameter measured was the ratio of peak flow following a 3-min period of ischaemia to basal skin blood flow. This parameter was measured before and after a 30-min period of raised venous pressure applied by inflating a tourniquet to 80 mmHg, a pressure similar to foot vein pressures experienced by patients with chronic deep vein insufficiency. Fifteen of the 17 limbs studied showed a fall in the peak flow: basal flow ratio after this period of venous hyper-

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tension, a statistically significant result.[22] This fall in microcirculatory reserve was not simply the result of a reperfusion injury, as we had recorded all peak flows on two consecutive occasions before and after the period of venous hypertension to eliminate this possibility.

Proponents of the oxygen diffusion barrier theory of venous skin damage point to low transcutaneous PO_2 ($tcPO_2$) values found in liposclerotic skin as supportive evidence.[23-26] $tcPO_2$ is conventionally recorded by heating the skin to 43–44°C to induce capillary 'arterialization' and maximum hyperaemia. This is because in neonates, for whom the technique was originally devised, $tcPO_2$ values recorded under these conditions approximate closely to the arterial PO_2 values.[27] This does not apply in adults largely because of increased skin thickness;[28] nevertheless, $tcPO_2$ continues to be measured in this way. Therefore, in order to draw conclusions about skin oxygenation at normal temperatures using this technique, it is necessary to assume that the degree of vasodilation occurring in the skin under study is indeed maximal. It is known that resting skin blood flow tends to be higher in liposclerotic skin than normal skin;[29,30] whether the microcirculation in such skin can increase its flow in response to heat to the same extent as normal skin is not known. Our finding of an impaired hyperaemic capability in liposclerotic skin in response to a short period of ischaemia led us to examine this question.

We measured laser-Doppler flux (LDF) and $tcPO_2$ on the same area of gaiter skin in 44 limbs, divided into controls, uncomplicated varicose veins and lipodermatosclerosis (LDS).[31] The laser-Doppler probe was held in a specially designed mount which incorporated a heating element, based on a design described by Rayman *et al.*[32] Basal LDF was recorded, following which the skin was heated to 43°C. The vasodilatory capacity of the skin was assessed by calculating the ratio of peak flow after heating to basal flow. The $tcPO_2$ was then measured in the usual way at the same site. Patients with LDS had both a significantly lower $tcPO_2$ level than normal limbs and a significantly lower peak:basal flow ratio. A regression analysis of the two parameters demonstrated a clear correlation between them ($r = 0{\cdot}524$, $P < 0{\cdot}001$). The implication of this study is that low $tcPO_2$ readings in venous disease reflect a reduced vasodilatory response to heat, not necessarily a low oxygen content at normal conditions: in short, it is skin perfusion which is impaired, not gas diffusion.

The aim of elucidating the precise pathogenetic mechanism at work in venous skin damage is, of course, to allow more rational treatment to be developed. The principles of compression and elevation have been appreciated for hundreds of years, but are clearly not effective in a large proportion of cases, as shown by the numbers of chronic leg ulcers. If inappropriate leucocyte activation is shown to be the fundamental event in the formation of venous ulcers, the way ahead will lie in the development of effective agents designed to stifle such activation. Already two agents which have profound effects on white cell function have been evaluated with encouraging results. Prostaglandin E_1 (PGE_1) has a number of profound effects on the microcirculation which include the reduction of white cell activation.[33] In a double-blind placebo-controlled trial of 44 proven venous ulcers, significantly more had healed after 6 weeks of treatment with PGE_1 than with placebo.[34] Pentoxifylline has also recently been shown to be beneficial in healing venous ulcers. This drug, better known for its use in peripheral arterial disease, was formerly thought to act by increasing red cell deformability and hence improving capillary perfusion. This explanation has been largely supplanted by the finding that it is a potent inhibitor of cytokine-mediated neutrophil activation.[35] Pentoxifylline also reduces white cell adhesion to endothelium and inhibits the release of free radicals produced by leucocyte respiratory bursts. A multicentre, placebo-controlled double-blind trial of 80 patients with venous ulceration showed that in 23 of 38 patients treated with 1200 mg pentoxifylline daily, their ulcers healed within 6 months, whereas this occurred in only 12 of the 42 patients treated with placebo. Conventional compression treatment was continued throughout the trial in both groups.[36]

These trials provide further support for the hypothesis that an inappropriate degree of white cell activity is involved in the aetiology of venous skin damage, and at the same time indicate a new therapeutic approach for the treatment of this common and debilitating disease.

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References

1 Callam MJ, Ruckley CV, Harper DR, Dale JJ. Chronic ulceration of the leg: extent of the problem and provision of care. *Br Med J* 1985; **290:** 1855–6.

2 Christopoulos D, Nicolaides AN, Szendro G. Venous reflux: quantification and correlation with the clinical severity of chronic venous disease. *Br J Surg* 1988; **75:** 352–6.

3 Sumner DS. Applied physiology in venous problems. In: *Surgery of the Veins.* (JJ Bergan, JST Yao, eds) Orlando: Grune & Stratton, 1985.

4 Gius JA. Arteriovenous anastomoses and varicose veins. *Arch Surg* 1960; **81:** 299–308.

5 Schalin L. Arteriovenous communications in varicose veins localised by thermography and identified by operative microscopy. *Acta Chir Scand* 1981; **147:** 409–20.

6 Haeger KHM, Bergman L. Skin temperature of normal and varicose legs and some reflections on the aetiology of varicose veins. *Angiology* 1963; **14:** 473–9.

7 Lindemayr W, Loefferer O, Mostbeck A, Partsch H. Arteriovenous shunts in primary varicosis: a critical essay. *Vasc Surg* 1972; **6:** 9–13.

8 Browse NL, Burnand KG. The cause of venous ulceration. *Lancet* 1982; **ii:** 243–5.

9 Layer GT, Stacey MC, Burnand KG. Stanozolol and the treatment of venous ulceration—an interim report. *Phlebology* 1986; **1:** 197–203.

10 McMullin GM, Watkin G, Coleridge Smith PD, Scurr JH. The efficacy of fibrinolytic enhancement with Stanozolol in the treatment of venous insufficiency. *Aust NZ J Surg* 1991; **61:** 306–9.

11 Michel CC. Aetiology of venous ulceration. *Br J Surg* 1990; **77:** 1071.

12 Cheatle TR, McMullin GM, Farrah J *et al.* Skin damage in chronic venous insufficiency: does an oxygen diffusion barrier really exist? *J Roy Soc Med* 1990; **83**: 493–4.

13 Schmid-Schonbein GW, Usami S, Skalak R, Chien S. The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels. *Microvasc Res* 1980; **19**: 45–70.

14 Lawrence MB, McIntire LV, Eskin SG Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. *Blood* 1987; **70**: 1284–90.

15 Moyses C, Cederholm-Williams SA, Michel CC. Haemoconcentration and accumulation of white cells in the feet during venous stasis. *Int J Microcirc: Clin Exp* 1987; **5**: 311–20.

16 Thomas PRS, Nash GB, Dormandy JA. White cell accumulation in dependent legs of patients with venous hypertension: a possible mechanism for trophic changes in the skin. *Br Med J* 1988; **296**: 1693–5.

17 Scott HJ, McMullin GM, Coleridge Smith PD, Scurr JH. Venous ulceration: the role of the white blood cell. *Phlebology* 1989; **4**: 153–9.

18 Coleridge Smith PD, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. *Br Med J* 1988; **296**: 1726–7.

19 Fagrell B Local microcirculation in chronic venous incompetence and leg ulcers. *Vasc Surg* 1979; **13**: 217–25.

20 Coleridge Smith PD, Scurr JH. Capillary function in patients with chronic venous insufficiency. *Br J Surg* 1988; **75**: 597

21 Scott HJ, Coleridge Smith PD, Scurr JH. Histological study of white blood cells and their association with lipodermatosclerosis and venous ulceration. *Br J Surg* 1991; **78**: 210–11.

22 Cheatle TR, Chittenden SJ, Coleridge Smith PD, Scurr JH. The effect on skin blood flow of short-term venous hypertension in normal subjects *Angiology* 1991; **42**: 114–22.

23 Clyne CAC, Ramsden WH, Chant ADB, Webster JHH. Oxygen tension on the skin of the gaiter area of limbs with venous disease. *Br J Surg* 1985; **72**: 644–7.

24 Kolari PJ, Pekanmaki K, Pohjola RT. Transcutaneous oxygen tension in patients with post-thrombotic leg ulcers: treatment with intermittent pneumatic compression. *Cardiovasc Res* 1988; **22**: 138–41.

25 Mannarino E, Pasqualini L, Maragoni G *et al.* Chronic venous incompetence and transcutaneous oxygen pressure. a controlled study. *Vasa* 1988; **17**: 159–61

26 Mani R, White JE, Barrett DF, Weaver PW. Tissue oxygenation, venous ulcers and fibrin cuffs. *J Roy Soc Med* 1989; **82**: 345–6.

27 Huch R, Lubbers DW, Huch A. Reliability of transcutaneous monitoring of arterial PO_2 in newborn infants. *Arch Dis Childhood* 1974; **49**: 213–8.

28 Falstie-Jensen N, Spaun E, Brochner-Mortensen J, Falstie-Jensen S The influence of epidermal thickness on transcutaneous oxygen pressure measurements in normal persons. *Scand J Clin Lab Invest* 1988; **48**: 519–23.

29 Whimster I. In. *Pathology and Surgery of the Veins of the Lower Limb* (Dodd H, Cockett RB, eds), 2nd edn. Edinburgh. Churchill Livingstone, 1976

30 Belcaro G. Microvascular evaluation by laser-Doppler flowmetry of the effects of Centellase in the treatment of severe venous hypertension and leg ulcers. *Phlebology* 1987; **2**: 189–95.

31 Cheatle TR, Stibe ECL, Shami SK *et al* The vasodilatory capacity of the skin in venous disease and its relationship to transcutaneous oxygen tension. *Br J Surg* 1991; **78**: 607–10

32 Rayman G, Williams SA, Spencer PD *et al.* Impaired microvascular hyperaemic responses to minor skin trauma in type 1 diabetes. *Br Med J* 1986; **292**: 1295–8.

33 Sinzinger H, Virgolini I, Fitscha P. Pathomechanisms of atherosclerosis beneficially affected by prostaglandin E1 (PGE1)—an update. *Vasa* 1989; Suppl. **28**: 6–13.

34 Rudofsky G. Intravenous prostaglandin E1 in the treatment of venous ulcers—a double-blind, placebo-controlled trial. *Vasa* 1989; Suppl. **28**: 39–43.

35 Sullivan GW, Carper HT, Novick WJ, Mandell GL. Inhibition of the inflammatory action of interleukin-1 and tumour necrosis factor (alpha) on neutrophil function by pentoxifylline. *Infect Immunol* 1988; **56**: 1722–9.

36 Colgan M-P, Dormandy JA, Jones PW *et al* Oxpentifylline treatment of venous ulcers of the leg. *Br Med J* 1990; **300**: 972–5.

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Discoid lupus erythematosus responsive to sulphasalazine

(ADONIS 000709639100218J)

SIR, Standard therapy for discoid lupus erythematosus (DLE) consists of potent topical steroids, with the attendant risk of steroid dermopathy. We report a patient who responded to sulphasalazine on two occasions with relapse on withdrawal of this systemic therapy.

A 57-year-old woman was diagnosed as having DLE 18 years previously. The facial eruption, characterized by red, scaly plaques that were associated with scarring, could only be controlled for 12 years by the daily application of a potent steroid, occasional intralesional steroids and a sun screen. There was thinning of the skin and telangectasia. A presumptive diagnosis of ulcerative colitis was made 6 years ago when she was abroad and she was treated with sulphasalazine 500 mg four times a day. Within 2 months she noted an improvement of her facial rash and was able to reduce the frequency of application of the topical steroid to twice a week. After 18 months, in the absence of proven colitis, her sulphasalazine was stopped, without a relapse of her bowel symptoms. However, there was a marked deterioration of her DLE, resulting in increased usage of topical steroid after 3 months. Following 2 years of poor control with topical therapy, the patient was recommenced on sulphasalazine 500 mg twice a day, with some improvement. Complete control was achieved after a further year when the dose was increased to 500 mg four times a day. The patient has remained on this dose of sulphasalazine for the past year, requiring only very occasional topical steroid therapy and has suffered no side-effects.

The spectrum of inflammatory disorders in which sulphasalazine has been used successfully continues to increase, extending from the licensed indications for inflammatory bowel disease and rheumatoid arthritis, to ankylosing spondylitis,[1] psoriatic arthropathy,[2] psoriasis,[3] other dermatoses[4] and alopecia universalis.[5] The potential steroid sparing effect of sulphasalazine in DLE exemplified by our patient, warrants further investigation.

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References

1 Nissila M, Lehtinen K, Leirisalo-Repo M *et al.* Sulphasalazine in the treatment of ankylosing spondylitis. *Arthritis Rheum* 1988; **31**: 1111–6.
2 Farr M, Kitas GD, Waterhouse L *et al.* Sulphasalazine in psoriatic arthritis: a double-blind placebo-controlled study. *Br J Rheumatol* 1990; **29**: 46–9.
3 Gupta AK, Ellis CN, Siegel MT *et al.* Sulphasalazine improves psoriasis. *Arch Dermatol* 1990; **126**: 487–93.
4 Schoch EP, McCuistion CH. The effect of salicylazosulfapyridine (azulfidine) on pustular acne vulgaris and certain other dermatoses. *J Invest Dermatol* 1955; **25**: 123–6.
5 Jawad ASM, Scott DGI. Remission of alopecia universalis during sulphasalazine treatment for rheumatoid arthritis. *Br Med J* 1989; **298**: 675.

Erythema nodosum and coeliac disease

(ADONIS 000709639100219W)

SIR, Erythema nodosum is a reaction pattern that has a number of possible causes. It has been reported in association with ulcerative colitis, regional enteritis and diverticulitis.[1–3] We report a patient who had erythema nodosum and coeliac disease.

A 17-year-old woman presented with a 6-month history of recurrent tender nodules on her lower legs that were associated with episodes of diarrhoea. She had been on no medication in recent months and a general physical examination was normal. A skin biopsy of a nodule showed the histology of a septal panniculitis that was consistent with the diagnosis of erythema nodosum. Direct immunofluorescence on the skin biopsy was negative. Other investigations showed that she had a megaloblastic anaemia with a haemoglobin of 10·7 g/dl and a MGV of 106. Serum folate was low being 1·4 mg/l. A biopsy taken from the distal duodenum showed complete loss of villi with hyperplasia of the crypts and an infiltrate of lymphocytes and plasma cells in the lamina propria. IgA anti-gliadin antibodies were positive in the serum, but no cryoglobulins were detected. A chest X-ray was normal and the concentration of angiotensin-converting enzyme was 38 U/ml (normal, <145 U/ml). No other cause for her erythema nodosum was found other than the coeliac disease. The skin lesions cleared completely without any recurrence after she was treated with a strict gluten-free diet. A year later the patient remained well and a recent small-bowel biopsy was normal.

This patient had erythema nodosum and coeliac disease. In coeliac disease there may be a variety of skin manifestations that include psoriasiform dermatitis, purpura and vasculitis as well as lesions of dermatitis herpetiformis.[4,5] The coexistence of erythema nodosum with coeliac disease has been reported but the cutaneous lesions were attributed to underlying sarcoidosis.[6,7] In our case, there was no evidence for sarcoidosis. However, the two conditions did appear to be related and it is possible that the erythema nodosum was caused as a result of increased intestinal permeability to endogenous or exogenous antigens.

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References

1 Sams WM, Winkelmann RK. The association of erythema nodosum with ulcerative colitis. *South Med J* 1968; **61**: 676–9.
2 Jacobs WH. Erythema nodosum in inflammatory diseases of the bowel. *Gastroenterology* 1959; **37**: 286–94.
3 Ruiz-Rodriguez R, Winkelmann RK. Erythema nodosum and diverticulitis. *Arch Dermatol* 1990; **126**: 1242–3.
4 Jones FA. The skin: a mirror of the gut. *Geriatrics* 1973; **28**: 75–81.
5 Meyers S, Dikman S, Spiera H *et al.* Cutaneous vasculitis complicating coeliac disease *Gut* 1981; **22**: 61–4.
6 Douglas JG, Gillon J, Logan RFA *et al.* Sarcoidosis and coelic disease: an association? *Lancet* 1984; **ii**: 13–5.
7 Lowe G, Johnston RN. Sarcoidosis and coeliac disease *Lancet* 1984; **ii**: 637.

Pseudocyst of the auricle. A birthday ear pull

(ADONIS 000709639100220Q)

SIR, We read with interest the paper by Devlin *et al.*[1] in which four children with severe atopic dermatitis developed pseudocysts of the auricles. Although the aetiology of this condition is obscure it has been suggested that trauma, albeit minor from scratching and rubbing, was a likely factor for this condition.[2–4]

We believe that the pseudocyst of the auricle is traumatic in origin, and this view was supported by two recent cases. Two unrelated Caucasian males, one aged 16 and the other 18 years, each developed a pseudocyst of an auricle 10–15 days after they had celebrated their birthdays. In many regions of Italy, it is customary on a birthday for the friends to twist and pull the ear as many times as the number of years of the birthday. It is often a violent action and at the very least induces erythema and oedema of the auricle.

In one of our cases, histological examination was carried out and this showed a pseudocyst, the cavity of which was lined by slightly hyalinized cartilage. There was a mixed inflammatory cell infiltrate consisting of lymphocytes and neutrophils and a moderate fibrosis of the perichondrial tissue. No blood was found in the perichondrium or in the cavity of the pseudocyst. In both cases a clear oil-like fluid was drained from the pseudocyst. There was slow resolution which was incomplete despite treatment with systemic anti-inflammatory drugs and topical steroid creams.

Our two cases suggest that trauma can be a factor in producing pseudocysts of the auricle and that pulling the ear on a birthday is perhaps a playful and dangerous custom in Italy.

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References

1 Devlin J, Harrison CJ, Whitby DJ, David TJ. Cartilaginous pseudocyst of the external auricle in children with atopic eczema. *Br J Dermatol* 1990; **122**: 699–704.
2 Lazar RH, Heffner DK, Hughes GB, Hyams VK. Pseudocyst of the auricle: a review of 21 cases. *Otoryngol Head Neck Surg* 1986; **94**: 360–1.
3 Grabski WJ, Salasche SJ, McCollough ML, Angeloni VL. Pseudocyst of the auricle associated with trauma. *Arch Dermatol* 1989; **125**: 528–30.
4 Glamb R, Kim R. Pseudocyst of the auricle. *J Am Acad Dermatol* 1984; **11**: 58–63.

Induction of ornithine decarboxylase in psoriatic skin

(ADONIS 000709639100223V)

SIR, In a recent publication[1] differences in methodology were proposed to explain the discrepancies between our earlier work[2] and their findings on the induction of ornithine decarboxylase (ODC) in normal and psoriatic skin. We wish to make the following observations. Because ODC is unstable, even at the temperature of liquid nitrogen, the storage conditions should be standardized. The tape stripping, for the induction of ODC, should also be standardized as well as the site for the biopsy. Both sun exposure and photochemotherapy can affect the levels of ODC.[3,4] In our study biopsies were taken from the abdomen and these were of full thickness skin to ensure that basal keratinocytes would be present, being the major source of ODC in viable epidermis. The levels of ODC activity in our study were expressed in different units, but on conversion were similar to those recently reported.[1]

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References

1 Arnold WP, Candida AEM, Van Hooijdonk *et al.* Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin. *Br J Dermatol* 1990; **123**: 581–5.
2 Bouclier M, Elbaze P, Milano G *et al.* Hyper-induction of skin ornithine decarboxylase activity in psoriatic patients. *Br J Dermatol* 1986; **115**: 193–8.
3 Lowe NJ, Breeding J. Retinoic acid modulation of ultraviolet light-induced epidermal ornithine decarboxylase activity. *J Invest Dermatol* 1982; **78**: 121–4.
4 Mizuno N, Kono T, Ishii M *et al.* Increase in the expression of the ornithine decarboxylase gene in mouse skin by ultraviolet light. *Arch Dermatol Res* 1989; **281**: 514–6.

Effect of corticosteroid therapy in the leucocyte adherence deficiency syndrome

(ADONIS 000709639100222I)

SIR, The leucocyte adherence deficiency syndrome (LAD) is a new genetic disorder that is characterized by recurrent cutaneous infections, severe periodontal disease and abnormalities in wound healing.[1] In this disorder the neutrophils demonstrate partial or complete deficiency in the membrane expression of the glycoproteins MO1, LFA1 and GP 150.95. Recently, three siblings were reported with congenital deficiency of leucocyte adherence glycoproteins and the lesions of pyoderma gangrenosum,[2] and in one of the patients prolonged therapy with corticosteroids did not prevent a fatal course. We reported in 1990 a case of a 30-year-old man with recurrent necrotic tissue infections that he had suffered from since childhood and with pyoderma-like lesions, impaired wound healing and periodontitis.[3] There was decreased adherence glycoprotein expression (MO1, LFA1 and GP 150,95) before and after stimulation with FMLP (f-Met-Leu-Phe). Functional abnormalities of the neutrophils were also found. This patient, in addition to having a large leg ulcer that had been present for over 2 years and with no tendency to heal, also had scars.

As a marked improvement had been observed in childhood following treatment with corticosteroids, he was given a course of prednisone in a dosage of 0·5 mg/kg per day for 3 months. The ulcer almost completely healed from initially 45 cm^2 before therapy to less than 5 cm^2.

Expression of the adherence glycoproteins (α and β chains of MO1 and LFA1 molecules) was determined by immunofluorescence flow cytometry before and after corticosteroid therapy using specific monoclonal antibodies (Table 1). Before treatment with corticosteroids the patient's polymorphonuclear neutrophils (PMN) expressed less than those of controls. FMLP stimulation did not enhance their expression. However, after 3 months of treatment with corticosteroids, although the PMN did not express more adherence glycoprotein, FMLP stimulation enhanced the expression in the same range as those from controls.

After 3 months of treatment with corticosteroids the PMN in our patient showed evidence of an intracellular pool of adherence glycoprotein that was expressed at the surface membrane and increased after stimulation with FMLP. We suggest that corticosteroid therapy in this syndrome can be beneficial in healing ulcerated lesions.

Table 1. Flow cytometry analysis of surface MAC1, LFA1 chain expression on patient granulocytes before and after corticosteroid therapy. Results are expressed as mean of fluorescence intensity for the patient and controls

	Before treatment		After treatment	
	Without FMLP	With FMLP	Without FMLP	With FMLP
MAC1				
Patient	81	88	80	101
Control	131	168	130	160
LFA1				
Patient	88	88	73	111
Control	112	114	110	109
β chain				
Patient	88	82	66	118
Control	127	128	115	119

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References

1 Anderson DC, Schmalsteig FC, Finegold MJ. The severe and moderate phenotypes of heritable MAC1, LFA1 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical feature. *J Infect Dis* 1985; **152:** 668–89.

2 Van de Kerkhof PCM, Weemaes CMR. Skin manifestations in congenital deficiency of leucocyte adherence glycoproteins (CDLG). *Br J Dermatol* 1990; **123:** 395–401.

3 Stalder JF, Pham Huu T, Vergracht A *et al.* Prednisone enhances wound healing and modifies polymorphonuclear expression in partial leucocyte adherence deficiency syndrome. *J Invest Dermatol* 1990; **94:** 402.

Topical treatment with the vitamin D_3 analogue MC903 improves pityriasis rubra pilaris: clinical and immunohistochemical observations

(ADONIS 000709639100221 5)

SIR, Topical treatment with the vitamin D_3 analogue MC903 has been shown to be effective in the treatment of psoriasis.[1,2] Pityriasis rubra pilaris is a condition where, like psoriasis, there is abnormal keratinization with increased epidermal proliferation and infiltration with T lymphocytes. It could also be responsive to topical treatment with MC903. We have recently treated a patient with this analogue of vitamin D_3 over a long-term period and he was initially diagnosed as having psoriasis but later during the course of therapy developed classical pityriasis rubra pilaris.

An 81-year-old man presented with an erythematous scaly rash that he had had for a number of years, and which had been diagnosed as psoriasis. He had responded to various treatments and was included in a trial to determine the long-term efficacy of MC903. The patient was on treatment for 10 months and the PASI score recorded at monthly intervals (Fig. 1). Punch biopsies were taken before and after 1, 2, 4, and

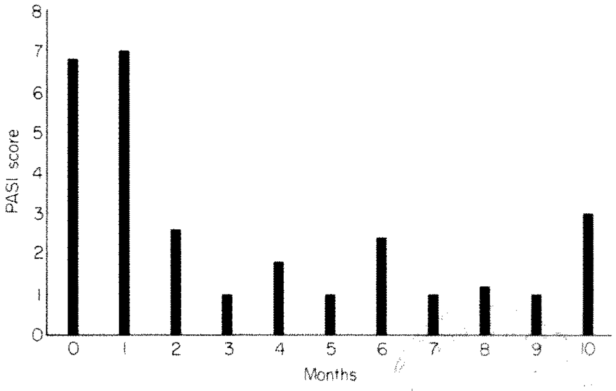

Figure 1. PASI scores before and at monthly intervals during treatment with MC903.

12 weeks of treatment. After 1 month of treatment there was a substantial improvement, but the clinical picture was now that of pityriasis rubra pilaris rather than psoriasis.

The results of immunohistochemical studies using monoclonal antibodies against a nuclear antigen present in cycling cells (Ki-67, Dakopatts, Copenhagen, Denmark)[3,4] and keratins 13 and 16 (Ks8.12, Sigma, St Louis, MO, U.S.A.) to assess epidermal proliferation[5,6] are summarized in Table 1. Before and after 1 week of treatment the nuclear binding of Ki-67 was substantially lower than that observed in untreated plaques of psoriasis.[7] After 2–4 weeks of treatment with MC903 the nuclear binding to Ki-67 was increased but still remained below that seen in psoriasis. No anti-elastase binding associated with polymorphonuclear leucocytes was observed in the biopsies before and during treatment with MC903. Ks8.12 binding (keratin 16 expression) was reduced after 2 weeks of treatment, and the number of T lymphocytes and of monocytes and macrophages, characterized by the monoclonal antibody WT14 (a gift from Dr Tax, Department of Medicine, University Hospital Nijmegen),[8] also showed a reduction within the first 2 weeks.

Table 1. Binding of monoclonal antibodies characterizing inflammation, epidermal proliferation and keratinization, before and at regular time intervals during treatment with MC903

Weeks	Ki-67*	Ks8.12	Anti-elastase	T11	WT14	OKT6
0	53·3	2–4	E:0 D:0	E:1–2 D:2–4	E:0 D:2	E:3–4 D:2–3
1	148·4	4	E:0 D:1	E:1–2 D:2–4	E:0 D:0	E:3–4 D:2–3
2	159·4	1–2	E:0 D:1	E:1 D:2–3	E:0 D:0	E:3 D:2–3
4	114·6	2	E:0 D:1	E:1 D:1–2	E:0 D:0	E:3–4 D:1–2
12	90·3	2	E:0 D:0	E:1 D:2–3	E:0 D:0	E:3–4 D:1–3
0–12	Up	Down	0	Down	Down	

* Ki-67, positive nuclei per mm length of epidermis.

E, epidermal binding: 0, no staining; 1, sporadic staining; 2, minimal staining; 3, moderate staining; 4, pronounced staining.

D, dermal binding: 0, no positive cells; 1, sporadic; 2, 1–25%; 3, 26–50%; 4, 51–75%; 5, 76–99%; 6, 100%.

Although there is an overlap between psoriasis and pityriasis rubra pilaris, it is usual to be able to separate the two conditions on clinical, histological and cell kinetic criteria.[9] In our case, the histology was that of pityriasis rubra pilaris, particularly in the absence of polymorphonuclear leucocytes in the skin biopsies. Treatment in our patient with topical MC903 resulted in an improvement and there was a reduction in the expression of keratin 16 and the number of T lymphocytes in the treated skin. This suggests that MC903 may be of use in the long-term treatment of disorders of keratinization apart from psoriasis.

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References

1 Kragballe K, Beck HI, Sogaard H. Improvement of psoriasis by a topical vitamin D_3 analogue (MC903) in a double-blind study. *Br J Dermatol* 1988; 119: 223–30.
2 Staberg B, Roed-Petersen J, Menne T. Efficacy of topical treatment in psoriasis with MC903, a new vitamin D_3 analogue. *Acta Derm Venereol (Stockh)* 1989; 69: 147–50.
3 Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983; 31: 13–20.
4 Van Erp PEJ, de Mare S, Rijzewijk JJ *et al.* A sequential double immunoenzymatic staining procedure to obtain cell kinetic information in normal and hyperproliferation epidermis. *Histochem J* 1989; 21: 343–7.
5 Huszar M, Gigi-Leitner O, Moll R *et al.* Monoclonal antibodies to various acidic keratins. *Differentiation* 1986; 31: 141–153.
6 Van Muijen GNP, Warnaar SO, Ponec M. Differentiation related changes of cytokeratin expression in cultured keratinocytes and in fetal, new born, and adult epidermis. *Exp Cell Res* 1987; 171: 331–45.
7 De Jong EMGJ, van de Kerkhof PCM. Simultaneous assessment of inflammation and epidermal proliferation in psoriatic plaques during long-term treatment with the vitamin D_3 analogue MC903: modulations and interrelations. *Br J Dermatol* 1991; 124: 221–9.
8 Bogman MJJT, van de Winkel JGJ, Hoitsma AJ *et al.* Diagnosis of renal allograft rejection by macrophage immunostaining with a CD14 monoclonal antibody, WT14. *Lancet* 1989, ii: 235–8.
9 Braun-Falco O, Rijckmanns F, Schmoeckel C, Landthaler M. Pityriasis rubra pilaris: a clinico-pathological and therapeutic study with special reference to histochemistry and radiography and electron microscopy. *Arch Dermatol Res* 1983; 275: 287–95.

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News and Notices

A practical course on keratinocyte cultures and their applications
Royal London Hospital, 9–11 October 1991

This course is for dermatologists, cell biologists, pharmacists and industrial representatives who wish to know how to grow keratinocytes and their clinical and biological relevance. There will be three half-day lecture sessions and two half-day practicals and so the number is limited to 30 attendees. Practical teaching will be in small groups and provide 'hands on' experience. For further information contact: Dr Irene Leigh, Experimental Dermatology, London Hospital Medical College, 56 Ashfield Street, London E1 2BL. Telephone 071-377-7724/7749.

International Symposium on Irritant Contact Dermatitis
Groningen, Holland, 3–5 October 1991

An international symposium on Irritant Contact Dermatitis will be held in Groningen. The symposium will cover the clinical, pharmacological, toxicological and biochemical aspects. There will be invited speakers, submitted presentations and posters as well as a social programme. The aim of the symposium is to stimulate discussion between all workers in the field of irritant contact dermatitis. For further information contact: Dr P. J. Coenraads or Dr P. G. M. van der Valk, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands. Telephone (31)-(0)50-612520; fax (31)-(0)50-612624.

Dystrophic Epidermolysis Bullosa Research Association

Applications are invited for research grants to be considered by the Medical and Scientific Advisory Panel of the Dystrophic Epidermolysis Bullosa Research Association (DEBRA). Application forms can be obtained from: DEBRA, 1 King's Road, Crowthorne, Berks RG11 7BG. Telephone: 0344-771961. The deadline for the receipt of completed applications is 31 October 1991.

British Society for Paediatric Dermatology
1–2 November 1991, Bristol

The Annual Scientific Meeting of the British Society for Paediatric Dermatology will be held at Burwalls on the Avon Gorge, Bristol on 1–2 November 1991. A major component of the meeting will be aspects of medical genetics in dermatology. For further information contact: Dr C.T.C.Kennedy, Department of Dermatology, Bristol Royal Infirmary, Bristol BS2 8HW. Telephone 0272-282785.

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The journal is a forum for new information about the direct and distant effects of electromagnetic radiation (ultraviolet, visible, and infrared) mediated through skin. The divisions of the editorial board reflect areas of specific interest: aging, carcinogenesis, immunology, instrumentation and optics, photosensitivity, pigmentation, and therapy. *Photodermatology, Photoimmunology & Photomedicine* includes original articles, reviews, communications, and editorials. Original articles may include the investigation of experimental or pathological processes in humans or animals *in vivo* or the investigation of radiation effects in cells or tissues *in vitro*. Methodology need have no limitation: rather, it should be appropriate to the question addressed. Thus, all contemporary physiologic, microscopic, and biochemical methodologies are welcomed.
Review articles, including new trends, are ordinarily solicited by the editors, although we invite suggestions from all readers. Communications are intended for the reporting of a special observation, either clinical or experimental, to address an hypothesis, to make a single point, or to pose a question.

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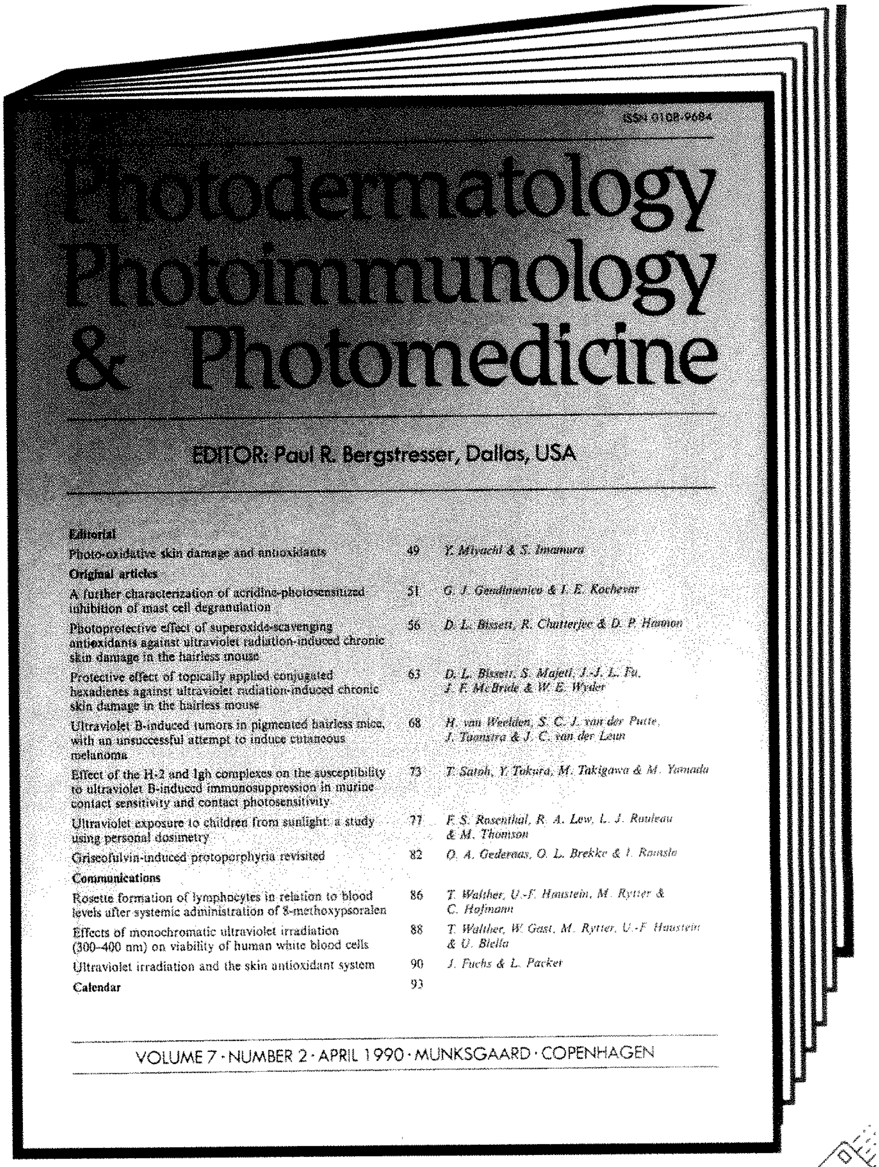

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nanogram(s) (10^{-9}g)	ng
picogram(s) (10^{-12}g)	pg
hours(s)	h
minute(s)	min
centimetre(s)	cm
second(s)	s
cubic millimetre(s)	mm^3
millimetre(s)	mm
millicurie(s)	mCi
milliequivalent	mmol
molar	mol/l
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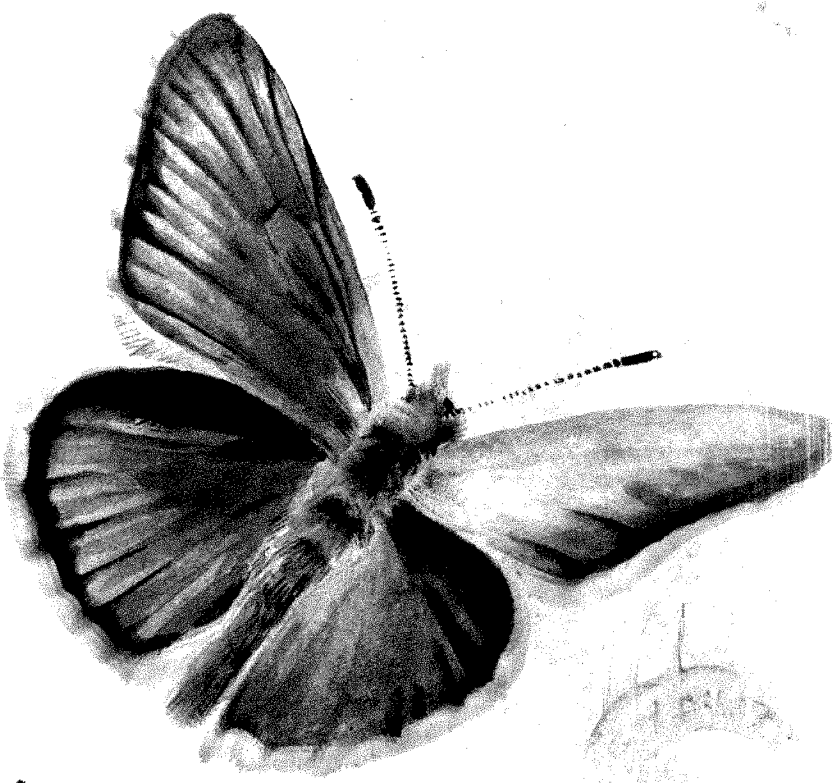

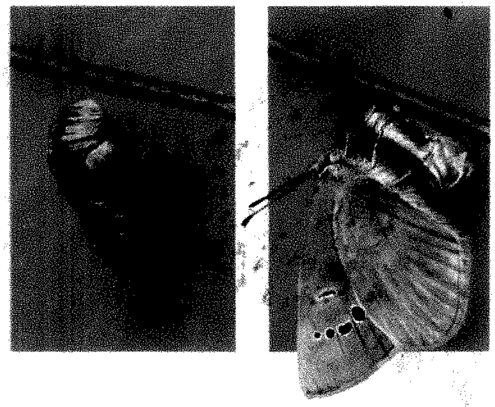

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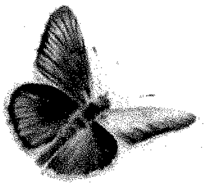

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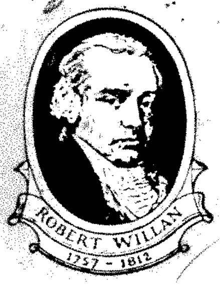

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This journal is printed on acid-free paper.

Printed in Great Britain at the Alden Press, Oxford

British Journal of Dermatology

Volume 125, Number 5, November 1991

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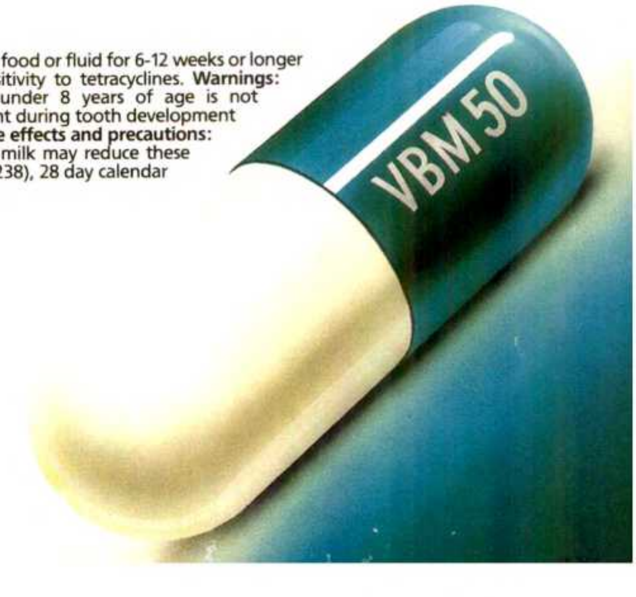
Reference
1. Harrison, P.V., (1988) Clin. Exp. Dermatol 13, 242-244



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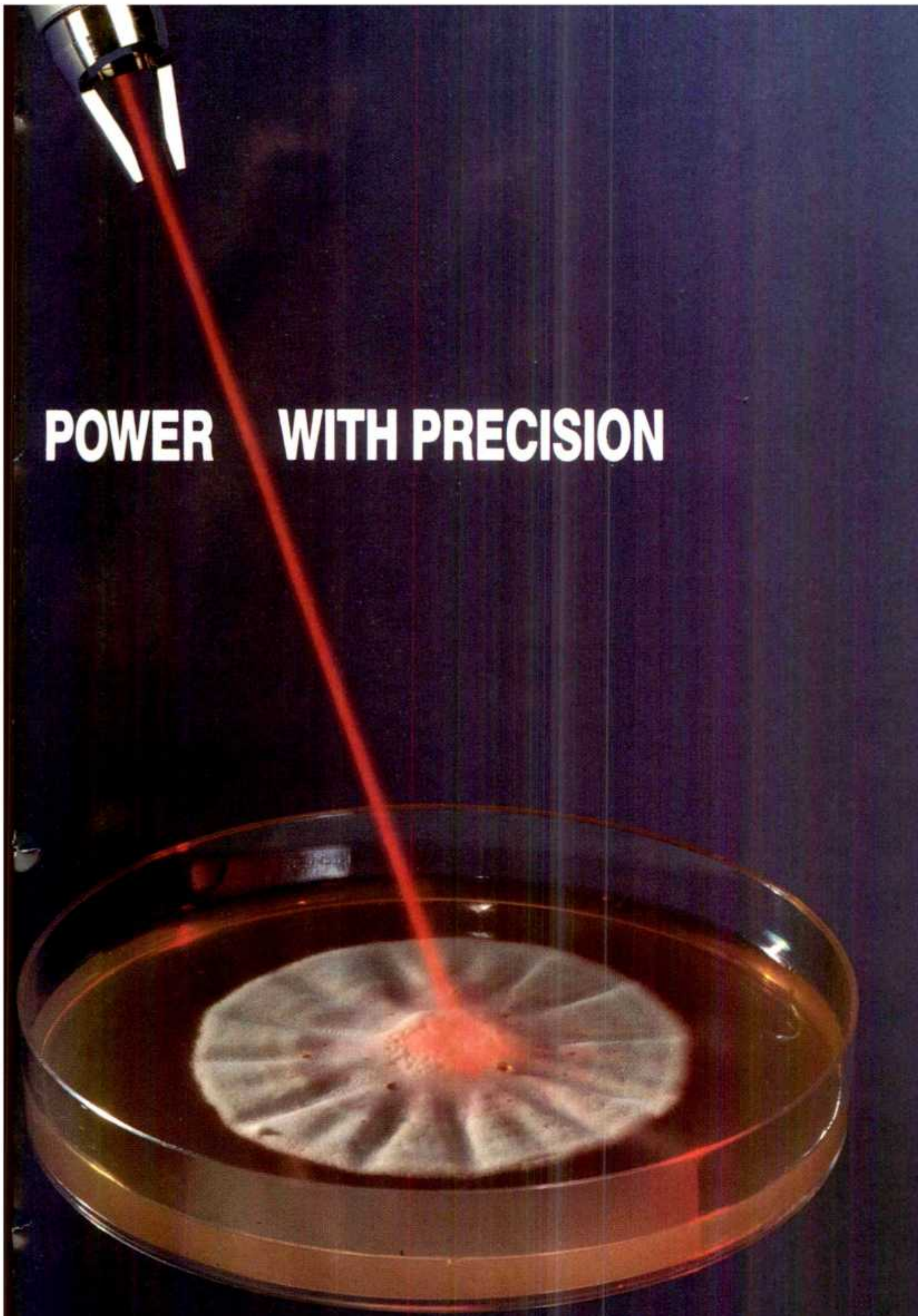

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References 1. Clayton YM. Clin Exp Dermatol, 1989, **14**, 2, 101-3. 2. Ryder NS. Antimicrob Agents Chemother, 1985; **27**, 252-6. 3. Schuster I. Xenobiotica, 1985, **15**, 529-46. 4. Schuster I, Ryder NS. J Dermatol Treat, 1990, **1**, (Suppl 2), 7-9. 5. Villars V, Jones TC. J Dermatol Treat, 1990, **1**, (Suppl 2), 33-8. 6. Savin R. Clin Exp Dermatol, 1989, **14**, 2, 116-19. 7. White JE, Perkins P, Evans EGV. Br J Dermatol, 1990, **123**, (Suppl 37), 30.

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Use of antibodies against the variable regions of the T-cell receptor α/β heterodimer for the study of cutaneous T-cell lymphomas

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Accepted for publication 10 June 1991

Summary

Recent studies have suggested that antibodies against the variable (V) regions of the T-cell antigen receptor (TCR) may be used as markers for clonality and malignancy in T-cell infiltrates. We have investigated this by examining biopsy samples from 45 patients with cutaneous T-cell lymphomas (CTCL) for reactivity with seven antibodies against different V-gene families on the TCR α/β heterodimer, i.e. ICI (Vβ5a), W112 (Vβ5b), OT145 (Vβ6a), 16G8 (Vβ8a), S511 (Vβ12a), F1 (Vα2a) and LC4 (αβVa). Serial biopsies were available in 13 patients and a total of 62 samples were studied. The neoplastic cells in five cases were positive for either Vβ5 (one case), Vβ6 (one case), Vβ8 (two cases) or Vβ12 (one case). In the remaining 40 cases, no staining was seen of the neoplastic cells. These findings indicate that while antibodies against the TCR V-regions may be used as clonotypic markers for certain T-cell neoplasms, there is as yet not a sufficient number of anti-TCR V-region antibodies available for the routine diagnosis of these conditions.

The T-cell antigen receptor (TCR) is a two-chain molecule consisting of α/β or γ/δ heterodimers. Both of these are associated with CD3 and consist of constant (C) regions and variable (V) domains. The V-domains are responsible for the binding of antigen to the TCR and are encoded by discontinuous gene segments which are juxtaposed (or rearranged) early in T-cell differentiation. Both variable (V), joining (J), and diversity (D) genes are implicated.[1]

The number of TCR V-genes is limited,[2–4] and it seems that much of the antigenic diversity of the TCR results from the use of different combinations of J and D genes and/or from the process known as junctional diversity, in which a random number of non-germline encoded nucleotides (N-nucleotides) are inserted between rearranged V, J and D genes.[5]

The fact that the number of TCR V-genes is limited has permitted the development of antibodies against different TCR V-gene families. It has been proposed that these antibodies may be used to distinguish between benign and malignant T-cell infiltrates.[6] In an attempt to elucidate this issue, we have examined biopsy samples from 45 patients with cutaneous T-cell lymphomas (CTCL) for reactivity with a panel of seven commercially available anti-TCR V-region antibodies.

Methods

Patients and biopsy samples

Skin biopsy samples were taken from 45 patients with CTCL. The diagnoses are summarized in Table 1 and were in accordance with standard criteria.[7] Serial samples and/or biopsies from different skin lesions were available in 13 cases, and a total of 62 samples were analysed.

Controls consisted of three biopsies from lymph nodes with benign hyperplasia and 12 biopsies from various benign inflammatory skin disorders, i.e. chronic eczema ($n=4$), psoriasis ($n=2$), lichen planus ($n=2$) and patch-test infiltrates ($n=4$) obtained at day 2 after exposure to either nickel or chromium.

Processing and staining of biopsies

The fresh biopsies were frozen in liquid nitrogen and

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Table 1. Reactivity of the neoplastic cells in 45 cases of CTCL with antibodies against framework determinants and variable (V) regions on the TCR/CD3 complex

Diagnosis	$V\beta 5a$ (ICI)	$V\beta 5b$ (W112)	$V\beta 6a$ (OT145)	$V\beta 8a$ (16G8)	$V\beta 12a$ (S511)	$V\alpha 2a$ (F1)	$V\alpha\beta a$ (LC4)	TCRβ (βF1)	TCRδ (TCRδ1)	CD3 (Leu 4)
MF, plaque ($n=8$)	0	0	0	0	0	0	0	7	0	8
MF, tumour ($n=10$)	0	0	0	0	1	0	0	7	0	10
Sézary syndrome ($n=8$)	0	0	0	0	0	0	0	8	0	8
CTCL, pleomorphic ($n=17$)	1	0	1	2	0	0	0	8	2	16
CLCL, anaplastic ($n=2$)	0	0	0	0	0	0	0	1	0	2
Total ($n=45$)	1/45	0/45	1/45	2/45	1/45	0/45	0/45	31/45	2/45	44/45

CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; TCR, T-cell antigen receptor.

stored at −80°C until processing. Cryostat sections (6 μm) were air-dried overnight at room temperature, fixed in acetone for 10 min and then stained with seven antibodies against different TCR V-gene families: ICI ($V\beta 5a$), W112 ($V\beta 5b$), OT145 ($V\beta 6a$), 16G8 ($V\beta 8a$), S511 ($V\beta 12a$), F1 ($V\alpha 2a$), and LC4 ($\alpha\beta Va$). All specimens were also examined for reactivity with βF1 (against a framework determinant of the TCR β chain),[8] TCRδ1 (against a framework determinant of the TCR δ chain),[9] CD3 (anti-Leu 4) and a range of other anti-T-cell antibodies, including CD4 (anti-Leu 3) and CD8 (anti-Leu 2).[7] All of the anti-TCR antibodies were obtained from T-Cell Science®. The remaining antibodies were purchased from Becton and Dickinson®.

In all cases, the staining was performed with the use of a three-stage immunoperoxidase method as previously described.[7]

Results

Benign lymphoid infiltrates

In skin and lymphoid organs with benign lesions, most of the T cells were positive with both CD3 and βF1. Only a few cells reacted with TCRδ1. Staining of the benign samples with seven anti-TCR V-region antibodies showed that very few (1–5%) T cells were positive. The TCR V-positive T cells were present in the dermis and paracortex and were scattered between numerous TCR V-negative T lymphocytes. Clustering of TCR V-positive T cells was not seen in any of the samples from the benign conditions.

In both skin and lymph nodes, dot-like, cytoplasmic positivity was seen with anti-$V\beta 6$ (OT145) in endothelial cells in some vessels resembling high endothelial venules. Cross-reactivity with other non-lymphoid cell types was not seen with any of the reagents.

Cutaneous T-cell lymphomas

The results from the staining of 62 skin biopsies from 45 patients with CTCL are summarized in Table 1. In 54 biopsies from 40 patients, staining with anti-TCR V-region antibodies was similar to that seen in the benign

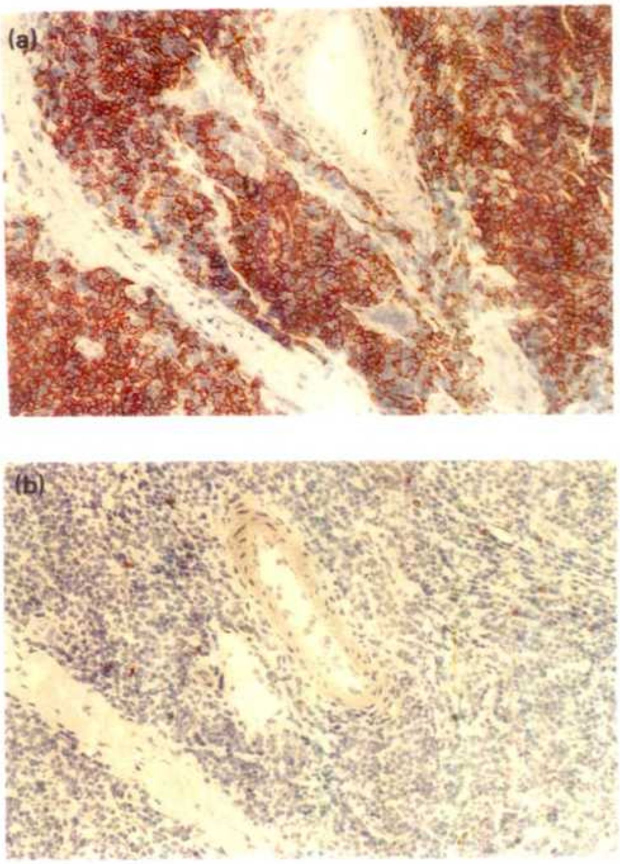

Figure 1. A CTCL of pleomorphic subtype. The neoplastic cells are (a) strongly positive for $V\beta 6$ (OT145, ×400) and (b) negative for other anti-TCR V-region antibodies, including $V\beta 8$ (16G8, ×400).

conditions described above. In these cases, no or only very few anti-TCR V-positive small lymphocytes (presumably residual reactive cells) were stained. No labelling was seen of the neoplastic cells.

In the remaining five cases, the neoplastic cells were positive for either Vβ5 (one case), Vβ6 (one case), Vβ8 (two cases) or Vβ12 (one case). Serial samples obtained during a follow-up period of 18 months were available in one of the cases. In all of these, the neoplastic cells expressed a similar phenotype and were strongly positive with an antibody (OT145) against the TCR Vβ6-family (Fig. 1).

All of the samples from the CTCL patients were also examined for reactivity with antibodies against framework determinants on the TCR/CD3 complex, including βF1, TCRδ1 and anti-Leu 4. These stainings showed that all of the five TCR V-region positive CTCL were negative for TCRδ1 and positive for both βF1 and CD3. Similar results were obtained in 26 of the 40 TCR V-negative cases. In the remaining 14 cases, the neoplastic cells were either negative for both βF1 and TCRδ1 (12 cases, Table 1) or positive for TCRδ1 and negative for βF1 (two cases, Table 1).

The two TCRδ1-positive CTCL were both negative for CD4 and CD8. The remainder showed staining for T-helper/inducer cells ($CD4^+$, $CD8^-$) in 30 cases and T-suppressor/cytotoxic cells ($CD4^-$, $CD8^+$) in four cases. Lack of expression of CD4 and CD8 (or co-expression of both molecules) was seen in nine cases. The expression of CD4 and CD8 did not vary between TCR V-positive and -negative cases of disease. Four of the TCR V-positive CTCL showed a helper T-cell phenotype and one case showed an aberrant T-cell phenotype with lack of expression of both CD4 and CD8.

Discussion

In this study, we have used a panel of seven antibodies against different V-gene families on the TCR α/β heterodimer to examine 62 skin biopsy samples from 45 patients with CTCL. Control samples from skin and lymphoid organs with benign lesions were also investigated.

The results confirm and extend previous reports and indicate that the staining of these reagents differs between benign and malignant T-cell infiltrates.[10] In benign lesions, only very occasional T cells are positive. This is in keeping with the fact that the V-regions in a benign T-cell population differ from one cell to another. By contrast, in a malignant T-cell clone, the V-regions are similar on all cells and if an appropriate antibody is available, all the cells will be strongly positive. These findings suggest that antibodies against the TCR V-regions may be used as markers for clonality (and malignancy) in T-cell infiltrates.[6] However, it is not clear whether there is a sufficient number of anti-TCR V-region antibodies available for the routine diagnosis of these conditions.

Three previous reports have addressed this issue. Clark *et al.* studied 24 T-cell malignancies with four antibodies and reported that the neoplastic cells in three cases were positive for either Vβ8 (two cases) or Vβ5 (one case).[6] No staining was seen of the neoplastic cells in the remaining 21 cases. Similar results were obtained by Charley *et al.* and O'Grady *et al.*[11,12] These authors used anti-TCR V-region antibodies similar to those in this study and reported that six of 38 T-cell neoplasms were positive for either Vβ5 (one case), Vβ6 (one case), Vβ8 (three cases) or Vβ5 plus Vβ8 (one case).

Ten of the 62 T-cell malignancies in these three studies[6,11,12] were CTCL. Three of these were positive for either Vβ5, Vβ8 or Vβ5 plus Vβ8. In the remaining seven CTCL patients, no staining was seen of the neoplastic cells. In another report[13] entirely different results were obtained. Among 16 CTCL patients 10 cases with histologically confirmed plaque or tumour lesions of mycosis fungoides were positive with an antibody (MX11) against the TCR Vβ8-family.[13] It was suggested that there is a preferential use of Vβ8 in CTCL, and that this might reflect similarities in the aetiology and/or pathogenesis of these malignancies.[13] The results obtained in our study do not support this view. Of 45 patients, only two cases were positive for Vβ8, an additional three cases were positive for either Vβ5, Vβ6 or Vβ12 and in the remaining 40 cases no staining was seen of the neoplastic cells. These results are very similar to those of the three studies mentioned above.[6,11,12]

The reason for the discrepancy between these studies is not clear. It has been shown that some T-cell malignancies do not express TCR molecules,[14,15] and one possibility is that differences may exist between TCR V-negative and -positive CTCL with respect to their expression of the TCR/CD3 complex. However, neither our results nor those of O'Grady *et al.*[12] support this view. In both studies all of the TCR V-positive and most of the TCR V-negative T-cell malignancies were positive for both CD3 and βF1 (against a framework determinant on the TCR β chain).[8] It is likely that these malignancies express TCR Vβ-molecules which are not detected with the anti-TCR V-region antibodies used in this and previous reports.

Another possibility is that CTCL may show geographical differences with respect to the expression of different TCR V-gene families. It is well known that many cases of CTCL are preceded by protracted, relatively unspecific skin lesions, and it has been suggested that chronic antigenic stimulation (resulting eventually in the emergence of a malignant T-cell clone) may be implicated in the pathogenesis of these conditions.[16] This implies that geographical differences in the exposure to environmental factors may result in the expression of different TCR V-genes in different CTCL populations. However, it remains to be established whether antigenic stimulation is involved in the pathogenesis of these conditions. In a recent publication the role of antigenic stimulation in CTCL has been questioned,[17] and further studies are needed.

It is concluded that antibodies against different V-gene families on the TCR α/β heterodimer may be used as markers for clonality and malignancy in certain T-cell neoplasms. The antibodies may provide useful information in individual lesions, but are of limited value for the routine diagnosis of these conditions. No more than 10% of the T-cell malignancies seen in this and other reports are positive with the currently available anti-TCR V-region antibodies, and the development of further reagents is required.

Acknowledgments

The expert technical assistance of Miss Annette Beth is gratefully acknowledged. This study was supported by the Harboe Foundation and the Aage Bang's Foundation. Financial support was also obtained from DAKO, Copenhagen, Denmark.

References

1 Cossman J, Uppenkamp M, Sundeen J *et al.* Molecular genetics and the diagnosis of lymphoma. *Arch Pathol Lab Med* 1988; **112:** 117–27.

2 Kimura N, Toyonaga B, Yoshikai Y *et al.* Sequences and diversity of the human T-cell receptor β chain variable region genes. *J Exp Med* 1986; **164:** 739–50.

3 Concannon P, Pickering LA, Kung P, Hood L. Diversity and structure of human T-cell receptor β-chain variable region genes. *Proc Natl Acad Sci USA* 1986; **83:** 6598–602.

4 Wilson RK, Lai E, Conconnon P *et al.* Structure, organization and polymorphism of murine and human T-cell receptor α and β chain gene families. *Immunol Rev 1988;* **101:** 149–72.

5 Siu G, Springer EA, Hedrick SM. The biology of the T-cell antigen receptor and its role in the skin immune system. *J Invest Dermatol* 1990; **94:** 91S–100S.

6 Clark DM, Boylston AW, Hall PA, Carrel S. Antibodies to T-cell antigen receptor beta chain families detect monoclonal T cell proliferation. *Lancet* 1986; **ii:** 835–7.

7 Ralfkiaer E, Wantzin GL, Mason DY *et al.* Phenotypic characterization of lymphocyte subsets in mycosis fungoides: comparison with large plaque parapsoriasis and benign chronic dermatoses. *Am J Clin Pathol* 1985; **84:** 610–19.

8 Brenner MB, McLean J, Scheft H *et al.* Characterization and expression of the human alpha/beta T-cell receptor by using a framework monoclonal antibody. *J Immunol* 1987; **138:** 1502–9.

9 Faure F, Jitsukawa S, Triebel F, Hercend T. Characterization of human peripheral lymphocytes expressing the CD3-gamma/delta complex with anti-receptor monoclonal antibody. *J Immunol* 1988; **141:** 3357–60.

10 Clark DM, Boylston AW. T-cell antigen receptor beta-chain variable region families: a study of their distribution in normal and reactive tissues. *J Pathol* 1989; **158:** 9–12.

11 Charley M, McCoy JP, Deng J-S, Jegasothy B. Anti-V region antibodies as 'almost clonotypic' reagents for the study of cutaneous T cell lymphomas and leukaemias. *J Invest Dermatol* 1990; **95:** 614–17.

12 O'Grady J, Krajewski AS, Ramage EF. Demonstration of clonality in T-cell lymphoma using an anti-T-cell receptor variable region antibody panel. *Histopathol* 1990; **17:** 553–6.

13 Jack AS, Boylston AW, Carrel S, Grigor I. Cutaneous T-cell lymphoma cells employ a restricted range of T-cell antigen receptor variable region genes. *Am J Pathol* 1990; **136:** 17–21.

14 Michie SA, Abel EA, Hoppe RT *et al.* Expression of T-cell receptor antigens in mycosis fungoides and inflammatory skin lesions. *J Invest Dermatol* 1989; **93:** 116–20.

15 Hastrup N, Pallesen G, Ralfkiaer E. Use of monoclonal antibodies for the diagnosis of T-cell malignancies. Applications and limitations. *Leukemia and Lymphoma* 1990; **2:** 35–45.

16 Tan RS-H, Butterworth CM, McLaughlin H *et al.* Mycosis fungoides—a disease of antigen persistence. *Br J Dermatol* 1974; **91:** 607–16.

17 Whittemore AS, Holly EA, Lee I-M *et al.* Mycosis fungoides in relation to environmental exposures and immune response: a case-control study. *J Natl Cancer Inst* 1989; **81:** 1560–7.

British Journal of Dermatology (1991) **125**, 413–418.

Antigenic profile of human acrosyringium

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Accepted for publication 28 May 1991

Summary

The demonstration of HLA-DR on human acrosyringium has led to the suggestion that eccrine epithelium, through its interaction with certain molecules, might play an active role in epidermal immune responses. An immunohistochemical study was undertaken to identify the antigenic profile of acrosyringium in normal skin and following the intradermal administration of a T-lymphocyte-derived cytokine, interferon gamma (IFN-γ). Acrosyringium in normal skin, in contrast to interappendageal epidermis, was found to lack CD1a$^+$ Langerhans cells. However, antigens CD36 (OKM5) and L1 (MAC387) were uniquely expressed by keratinocytes immediately adjacent to the distal portion of acrosyringium. Constitutive expression of each class II MHC antigen, namely HLA-DR, DP and DQ was observed on luminal acrosyringial cells. EBM11 antigen (CD68), a mononuclear cell determinant, was similarly expressed on acrosyringial epithelium in normal skin. Following intradermal administration of IFN-γ, intercellular adhesion molecule-1 (ICAM-1) (CD54) was induced on acrosyringial epithelium and the expression of HLA-DR was intensified. A range of other markers including CD3, CD4, CD8, CD11a, CD11b and CD15 were not expressed by acrosyringium either in normal skin or after administration of IFN-γ.

Expression of antigens associated with cell-mediated immune mechanisms on acrosyringium is consistent with the hypothesis that it may have an immunological role in epidermal immune responses.

Acrosyringium is the intraepidermal portion of the eccrine sweat gland and acts as the terminal conduit for the passage of eccrine secretions. Eccrine glands perform two main functions, namely thermoregulation and odour production, and are distributed over the entire surface of the skin except on mucous membranes. The density of eccrine structures varies according to site from about 620 cm^2 on the palms and soles to 64 cm^2 on the trunk.[1] Acrosyringial epithelium is embryologically derived from keratinocytes[2] but lacks a stratum corneum, the epidermal layer which has been shown to be the principal component resisting penetration by physical and chemical environmental agents.[3]

Although the importance of appendageal structures as routes of entry into the epidermis remains controversial, eccrine glands represent a potentially large area of epithelium with which molecules from the external environmental might directly interact. For example, inflammation originating around sweat ducts, a feature of irritant and contact dermatitis, may be a reflection of such interactions.[4] Furthermore, in contrast to normal interappendageal epidermis, constitutive expression of class II major histocompatibility (MHC) antigen HLA-DR, which plays a critical role in cell-mediated immune responses, has been demonstrated on acrosyringial epithelium in normal skin.[5]

These findings have led to the hypothesis that eccrine epithelium might have an immunological role in the epidermis, for example through its interaction with external agents. Many studies suggest an important pathophysiological role for activated T-lymphocyte-derived cytokine interferon gamma (IFN-γ) in cutaneous inflammation, including contact dermatitis.[6] Following intradermal administration of IFN-γ, T cells accumulate in the skin and keratinocytes express HLA-DR and ICAM-1 (CD54), two molecules central to cell-mediated responses.[7,8] The present study was undertaken firstly to identify the acrosyringial expression in normal skin of certain antigens that are requisite for immune competence and secondly to identify the effect of IFN-γ *in vivo* on human acrosyringium.

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Methods

Normal skin

Following local anaesthesia with 1% lignocaine, elliptical skin biopsies of normal skin were obtained from the abdomen of 15 adult male volunteers. Each biopsy was bisected and half was fixed in formalin prior to embedding in paraffin and the other half embedded in OCT, snap frozen and then stored in liquid nitrogen.

In vivo IFN-γ

Nine normal adult male volunteers received intradermal injections of 10 μg recombinant human IFN-γ (rIFN-γ; specific activity = 10^7/mg; donated by Biogen Incorporated, Boston, MA, U.S.A.), diluted in 0·1 ml sterile water, into the left buttock on 3 consecutive days. A control area, on the right buttock was injected with diluent only. Punch biopsies (4 mm) were obtained on day 6 (i.e. 72 h after the final injection) from both active and control sites and processed as outlined above.[7]

Immunohistochemistry

When required, 5 μm cryostat sections were obtained and transferred to 3-amino propyltriethoxysilane-coated glass slides. Each slide was air-dried for 10 min, fixed in acetone for 10 min and then labelled with the relevant monoclonal antibody (Table 1) using a peroxidase–antiperoxidase (PAP) immunohistochemical technique.[9] Staining was visualized using the chromogen 3,3′-diaminobenzidine with haematoxylin as the counterstain. For staining with MAC387 antibody, paraffin sections were dewaxed in xylene, dehydrated through graded ethanol, fixed and labelled as above. Appropriate controls were included by substitution of the primary monoclonal antibody with an antibody of irrelevant specificity but of the same IgG isotype. Staining intensity was graded on a scale 0 to + + + + by two independent observers.

Results

Normal skin

In sections from normal skin ($n = 15$) and in control biopsies (diluent only) ($n = 9$) epidermal staining for each class II MHC determinant was confined to the acrosyringial epithelium and epidermal Langerhans cells, which were also CD1a (OKT6) positive. Some sections ($n = 10/24$) also demonstrated class II antigen expression on dermal sweat ducts and glands, but staining was consistently less intense. Differential expression of class II MHC antigens on acrosyringial epithelium was observed. HLA-DR was invariably expressed ($n = 24/24$) while HLA-DP ($n = 19/24$) and HLA-DQ ($n = 14/24$) were observed in most sections examined. A differential staining intensity was also consistently noted with each class II MHC determinant (Fig. 1): HLA-DR > HLA-DP > HLA-DQ. Constitutive expression of the antigen EBM11 (CD68) was also seen on acrosyringial epithelium ($n = 24/24$), but not on dermal ducts or sweat glands.

Table 1. List of antibodies used and staining pattern of acrosyringium in normal skin and following administration rIFN-γ

Antibody	CD	Specificity	Normal skin	Control skin	Following rIFN (30 μg)	Source
OKM5	36	Monocyte/platelet	+	+	+	Ortho
MAC387		Calgranulin, L1 antigen	+	+	+	Dako
L243		HLA-DR	+ + +	+ + +	+ + + +	Becton Dickinson
B7/21		HLA-DP	+ +	+ +	+ +	Becton Dickinson
SK10		HLA-DQ	+	+	+	Becton Dickinson
RR1/1	54	ICAM-1	–	–	+ +	T. Springer
OKM1	11b	MAC1, C3bi receptor	–	–	–	Ortho
Anti LFA-1	11a	LFA-1	–	–	–	Dako
Leu M1	15	Granulocyte/monocyte	–	–	–	Becton Dickinson
EBM11	68	Monocyte/macrophage	+ + +	+ + +	+ + +	Dako
OKT6	1a	Langerhans' cells	–	–	–	Ortho
Leu 1	3	Pan T cell	–	–	–	Becton Dickinson
Leu 2a	8	Suppressor cell	–	–	–	Becton Dickinson
Leu 3a	4	Helper cell	–	–	–	Becton Dickinson

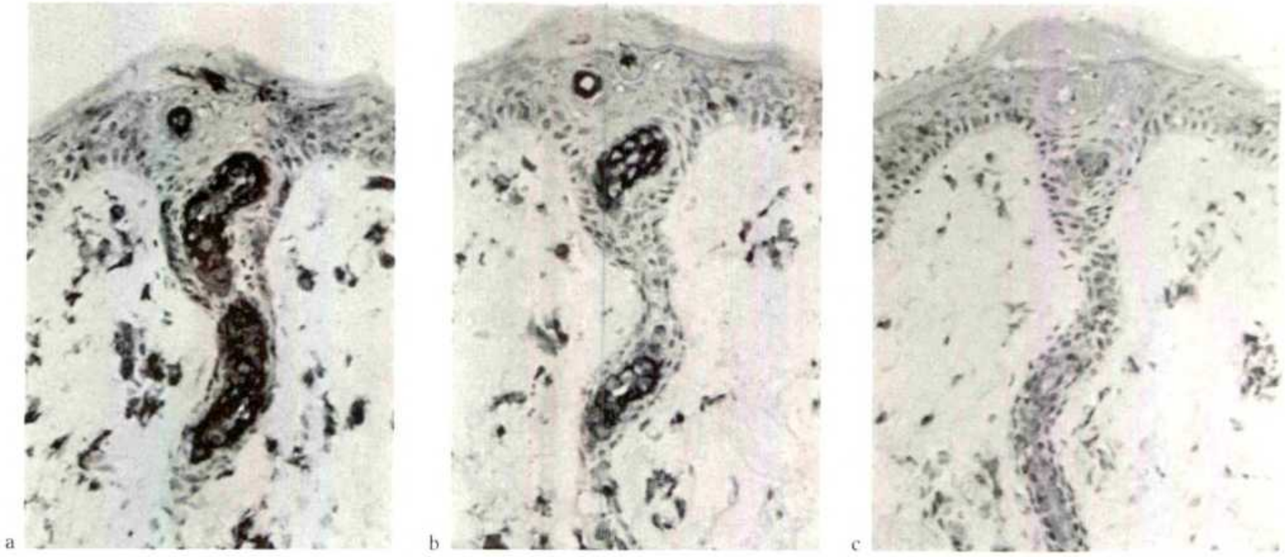

Figure 1. Constitutive expression of MHC class II antigens on acrosyringial epithelium in normal skin with differential staining intensity of each antigen (a) HLA DR, (b) HLA DP, (c) HLA DQ (× 100).

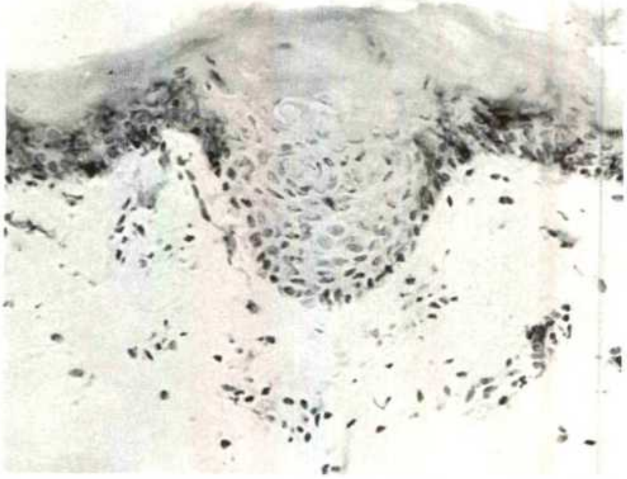

Figure 2. CD1a$^+$ Langerhans cells are present throughout the epidermis but absent around the acrosyringium (× 150).

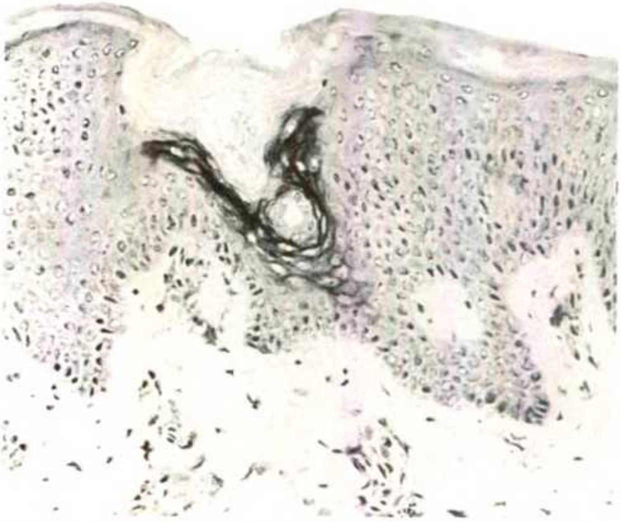

Figure 3. CD36 (OKM5) is expressed on keratinocytes immediately surrounding the intraepidermal opening of the acrosyringium but not on acrosyringial epithelium (× 150).

In contrast to interfollicular epidermis, an absence of CD1a$^+$ Langerhans cells was observed around acrosyringium (Fig. 2). Keratinocytes surrounding the distal portion of acrosyringium, but not acrosyringial epithelium, showed strong staining for CD36 (OKM5) ($n=24/24$) (Fig. 3). In paraffin sections ($n=6/24$) positive cytoplasmic staining for L1 (MAC387) was also seen in a similar distribution to CD36 (Fig. 4). The staining pattern for CD36 and L1 was thus consistently different from that for class II MHC and CD68. None of the above antigens were expressed on normal interfollicular keratinocytes. All other antigens (Table 1), including ICAM-1, were absent on acrosyringial epithelium.

Effect of IFN-γ

In sections from sites receiving rIFN-γ ($n=9$), staining intensity for HLA-DR antigen was increased on acrosyringial epithelium while HLA-DP, HLA-DQ and CD68 were unchanged. In addition, HLA-DR expression by

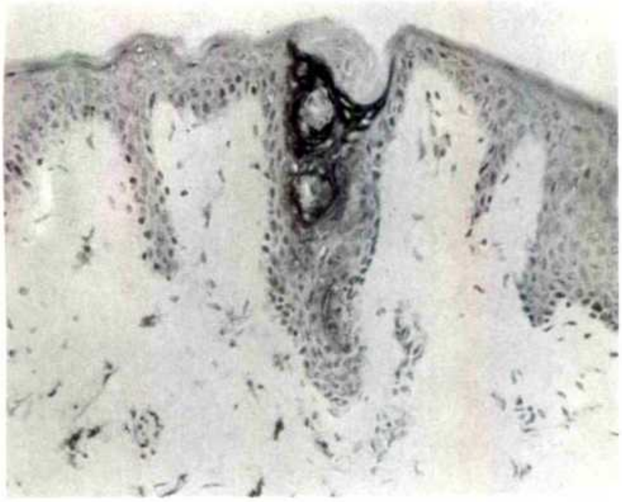

Figure 4. Cytoplasmic L1 (MAC387) expression surrounding the intraepidermal opening of acrosyringium is similar to CD36 expression (see Fig. 3) (× 100).

keratinocytes throughout the thickness of the epidermis was also seen, as previously described.[7] ICAM-1 expression was induced on both epidermal and acrosyringial keratinocytes (Fig. 5). CD36 and L1 expression was unchanged; CD1a, CD3, CD4, CD8, CD11a, CD11b and CD15 were not induced.

Discussion

This study of normal skin has demonstrated differential expression of class II MHC antigens and constitutive expression of CD68 on acrosyringial epithelium. In contrast, CD68 was not expressed and class II MHC expression was less intense on dermal and glandular epithelium. Expression of CD36 and L1 antigens was confined to epidermal keratinocytes immediately adjacent to the distal portion of acrosyringium. In addition, ICAM-1 expression was induced on acrosyringial epithelium following IFN-γ. Expression of antigens associated with cell-mediated immune mechanisms on eccrine epithelium suggests that acrosyringium may have an immune-competent role in epidermal immune responses.

The possibility that positive staining is due either to cross reactivity of epidermal proteins or adsorption of excreted proteins on to eccrine epithelium is unlikely. Use of monoclonal antibodies to the same antigens, HLA-DR,-DP,-DQ and ICAM-1 for example, developed in different laboratories yielded similar results (unpublished observation). Although human sweat is known to contain interleukin-1 (IL-1),[10] other immune-related molecules have not been demonstrated in sweat and adsorption of intraluminal molecules on acrosyringial

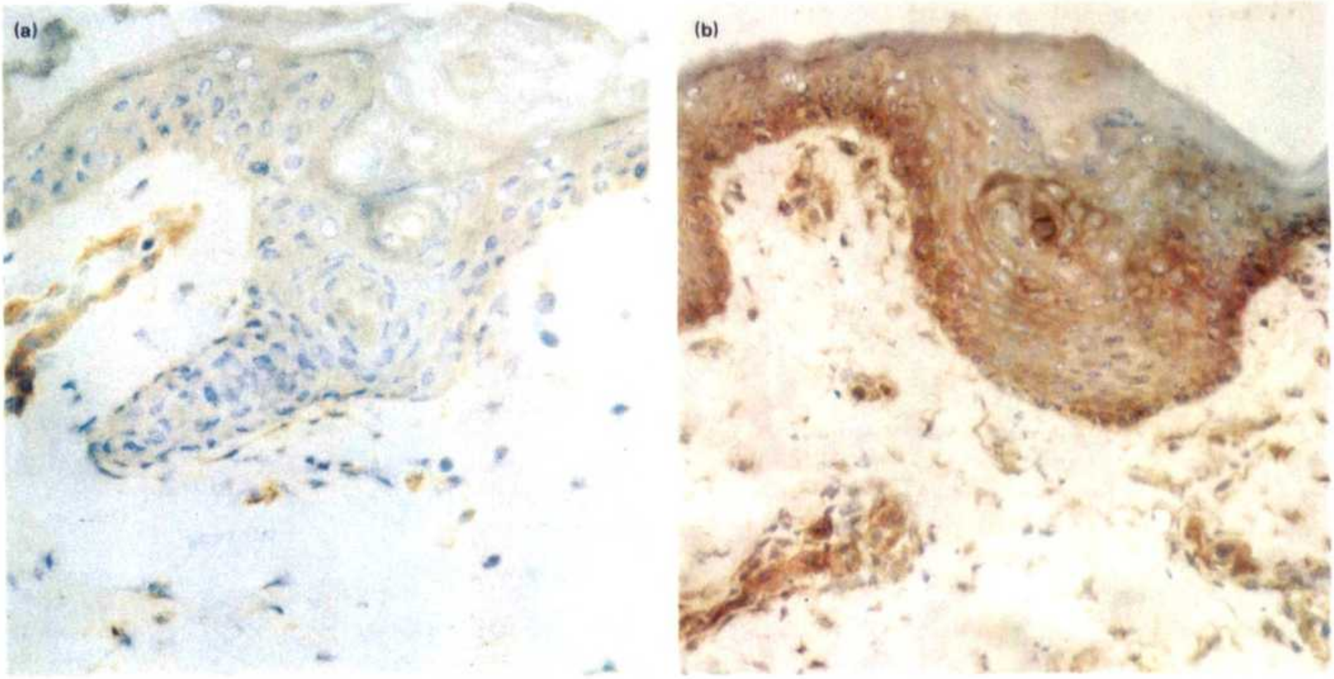

Figure 5. (a) ICAM-1 expression is confined to vascular endothelial cells in normal skin. Epidermal staining is absent (× 150) (b) Acrosyringial and epidermal keratinocyte expression of ICAM-1 following *in-vivo* IFN-γ (× 150).

epithelium is unlikely to account for the pattern of antigen expression observed in this study. For example, CD36 and L1 antigens are not expressed on luminal cells (Fig. 3) and differential expression of MHC antigens and induction of ICAM-1 following IFN-γ are events typically observed on epidermal keratinocytes in inflammation.[7,8] A similar pattern of expression on acrosyringium is likely to represent a pathophysiological response.

Eccrine sweat ducts are thought to be involved in percutaneous absorption of certain molecules.[3] When nickel is applied directly to normal skin, it is detectable in small amounts in the stratum corneum and is retained in the acrosyringial lumen.[11] Immunohistochemical studies have demonstrated that an influx of T lymphocytes and induction of HLA-DR on keratinocytes around sweat ducts is a feature of both allergic contact and irritant dermatitis,[4] suggesting that agents applied directly to skin can penetrate the epidermis through the acrosyringium and initiate an inflammatory reaction locally.

Expression of the surface antigen CD36 and cytoplasmic antigen L1 on keratinocytes is a feature of disordered epidermis common to many inflammatory dermatoses including psoriasis, contact dermatitis and lichen planus.[12] Constitutive expression of these antigens by keratinocytes surrounding the distal portion of acrosyringium could represent a non-specific reaction of these cells to injurious environmental stimuli,[13] particularly as these antigens are not expressed on keratinocytes surrounding more proximal parts of the sweat-gland apparatus. In support of this hypothesis, L1 staining is found on normal human mucosa which is subject to repeated mechanical trauma[14] but is absent on normal epidermis which is protected by stratum corneum except around the acrosyringial lumen.

It has now been shown that keratinocytes can respond directly to many agents known to cause cutaneous inflammation, including retinoic acid, phorbol esters and urushiol, by the induction and release of proinflammatory mediators, including IL-1, IL-8 and tumour necrosis factor-alpha (TNF-α).[15] Cultured keratinocytes have also been shown to produce IL-1 in response to nickel sulphate at concentrations lower than that required to induce nickel contact sensitivity *in vivo*.[16] Recent immunohistochemical studies have demonstrated active IL-1 production by acrosyringial epithelium in the distal portion of the eccrine gland,[10] suggestive of cytokine induction subsequent to injury from external agents.

Although synthesis of IFN-γ by keratinocytes has not been demonstrated, the absence of CD1a$^+$ Langerhans cells around the acrosyringium in normal skin would be consistent with local production of IFN-γ, presumably by activated T lymphocytes. A similar depletion of CD1a$^+$ Langerhans cells is observed as a direct effect of IFN-γ on normal human epidermis.[7] Differential expression of class II subregion products demonstrated on acrosyringium in this study further supports this hypothesis, as a similar pattern of class II MHC antigen expression is observed on cultured keratinocytes after exposure to IFN-γ[17] and is also seen on keratinocytes in inflammatory cutaneous diseases where IFN-γ is thought to play a major role.[18]

This study demonstrates antigen expression on acrosyringial epithelium consistent with the hypothesis that these specialized keratinocytes are immune-competent cells. Although the putative immunological role of the acrosyringium in the epidermis is unknown, it is well placed to mediate inflammatory reactions arising through percutaneous absorption of harmful environmental agents.

References

1 Sato K, Dobson RL. Regional and individual variations in the function of the human eccrine sweat gland. *J Invest Dermatol* 1970; **54:** 443–50.

2 Christophers E, Plewig G. Formation of the acrosyringium. *Arch Dermatol* 1973; **107:** 378–82.

3 Scheuplein RJ, Blank IH. Permeability of the skin. *Physiol Rev* 1971; **51:** 702–5.

4 Gawkrodger D, Carr M, McVittie E *et al.* Keratinocyte expression of MHC class II antigens in allergic sensitization and challenge reactions in irritant contact dermatitis. *J Invest Dermatol* 1987; **88:** 11–15.

5 Murphy G, Shephard R, Harrist T *et al.* Ultrastructural documentation of HLA-DR antigen reactivity in normal human acrosyringial epithelium. *J Invest Dermatol* 1983; **81:** 181–3.

6 Nickoloff BJ. The role of gamma interferon in epidermal trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. *Arch Dermatol* 1988; **124:** 1835–43.

7 Barker JNWN, Allen MH, MacDonald DM. Alterations induced in normal human skin by *in vivo* interferon gamma. *Br J Dermatol* 1990; **122:** 451–6.

8 Barker JNWN, Allen MH, MacDonald DM. The effect of *in vitro* interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. *J Invest Dermatol* 1989; **93:** 439–42.

9 Holden CA, MacDonald DM. Immunoperoxidase techniques in dermatopathology. *Clin Exp Dermatol* 1983; **8:** 443–57.

10 Reitamo S, Anttila H, Didierjean L, Saurat J-H. Immunohistochemical identification of interleukin 1 in human sweat gland apparatus. *Br J Dermatol* 1990; **122:** 315–23.

11 Wells G. Effects of nickel on the skin. *Br J Dermatol* 1956; **68:** 237–42.

12 Barker JNWN, Markey AC, Allen MH, Macdonald DM. Keratinocyte expression of OKM5 antigen in inflammatory cutaneous disease. *Br J Dermatol* 1989; **120:** 613–18.

13 Barker JNWN, Allen MH, Griffiths CEM *et al.* Expression of the

British Journal of Dermatology (1991) **125**, 419–425.

Sequence of morphological events during topical application of retinoic acid on the rhino mouse skin

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Accepted for publication 21 May 1991

Summary In a study of the comedolytic action of retinoids on the epidermal pseudocomedones of rhino mouse skin, the earliest changes and the sequence of events induced by the treatment were investigated. Rhino mice were treated topically with all-*trans* retinoic acid and at various time intervals from day 0 to 3 weeks, histological and ultrastructural studies as well as quantification of mast cells were performed. The earliest changes occurred in the dermis after 2 days of treatment and were characterized by degranulation of mast cells, clumping and association of Langerhans cells and lymphocytes. During the second week of treatment there was hyperplasia, hypergranulosis and an increase in the number of membrane coating granules with disorganization of the horny layer both of the epidermis and the follicular epithelium. These changes in the proliferation and the differentiation of keratinocytes resulted in a comedolytic effect. Associated with these epidermal changes there were changes in the dermis with an increased number of mast cells and a decreased number of Langerhans cells.

The skin of the adult rhino mouse (hr^{rh} hr^{rh}) is characterized by the utriculi that are derived from the infundibular zone of the initial follicular units.[1] These are histologically similar to retentional acneic lesions, such as comedones. These rhino mice are used as an *in-vivo* model to evaluate the potential comedolytic action of new drugs for the treatment of acne.[2–4] Retinoids produce a good comedolytic effect by reducing the number, size and diameter of the pseudocomedones.[2,3] We have shown that this comedolytic effect is related to changes in the proliferation and the differentiation of keratinocytes situated both in the epidermis and in the epithelial wall of pseudocomedones.[4] It is now well known that retinoic acid influences keratinocyte differentiation and proliferation.[5–8] Some authors have also reported effects on the dermal extracellular matrix,[9,10] and others have examined modifications of the dermal inflammatory components in disorders such as psoriasis.[11] However, no kinetic experiments have so far been reported.

The aim of this study was to determine the sequence of morphological events which occur in the different compartments of the rhino mouse skin, during treatment with a topical retinoid.

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Methods

Animals and treatment

Three groups of 6-week-old male and female rhino mice (hr^{rh} hr^{rh}) (CNRS, La Source, 45000 Orleans France) were used. In Groups 1 and 2 the animals were treated over the entire dorsal surface with 50 μl of either the drug preparation (all-*trans* retinoic acid gel at 0·025% w/w (Aberel® gel, Cilag Laboratories) or acetone, once a day, 5 days per week. Group 3 was composed of non-treated controls.

Biopsies

After 2, 3, 5, 8, 10, 12, 15, 17 and 19 days of treatment, three animals in each group were sacrificed by cervical dislocation. Punch biopsies (6 mm) were taken from the treated zones and cut into three pieces which were immersed in formalin, Karnovsky's solution, or frozen in liquid nitrogen.

Histology

After fixation in formalin, samples were embedded in paraffin and 4-μm vertical skin sections were stained with haematoxylin–phloxin–safran.

Electron microscopy

Biopsies were fixed for 4 h at room temperature in Karnovsky's solution (2% w/v paraformaldehyde, 2·5% w/v glutaraldehyde in 0·1 M cacodylate buffer, pH 7·4), then washed in 5% w/v sucrosed cacodylate buffer, post-fixed in 1% OsO_4, dehydrated and embedded in Epon 812. Semithin and ultrathin sections were cut and mounted on grids which were then observed with a Jeol 1200 EX electron microscope operating at 80 kV.

Some samples were prepared for scanning-electron microscopy (SEM). After fixation and dehydration, they were critical-point dried and coated with gold. Specimens were observed with Jeol T300 scanning-electron microscope operating at 25–30 kV.

Quantification of dermal Langerhans cells

The number of Langerhans cells per 50-μm^2 area of dermis was determined in sections from six non-treated animals and from five animals treated with the drug. Statistical analysis was performed using the Wilcoxon two-sided test for comparison of treated and non-treated skin.

Mast-cell labelling

Frozen samples were embedded in Tissue Tek (Miles Laboratories). Vertical cryosections (5 μm) were fixed in 4% w/v paraformaldehyde solution in phosphate-buffered saline (PBS, pH 7·2) for 10 min, washed with PBS, incubated with 1/500 FITC-avidin (Miles-Yeda LTD) for 60 min as previously described,[12] washed three times in PBS and mounted. Slides were examined by fluorescence microscopy with an Axiophot Zeiss microscope. The number of cells was determined using an ocular grid at × 20 magnification, counting the mast cells in five 0·5-mm^2 fields in each section. Three alternate serial sections were observed for each animal, and the mean value calculated. The standard error of the mean was evaluated for each group of three animals.

Results

In acetone-treated skin, no significant modifications were observed.

Histology

After 5–8 days of treatment the first effects were noted in the epidermis and in the epithelial wall of pseudocomedones with an enlargement of the intercellular space which disappeared after day 12. Simultaneously there was comedolysis and this increased up to day 17, when there was a decrease in the size and diameter of the utriculi and expulsion of follicular plugs occurred (Fig. 1). Between days 5 and 12, hyperplastic features were

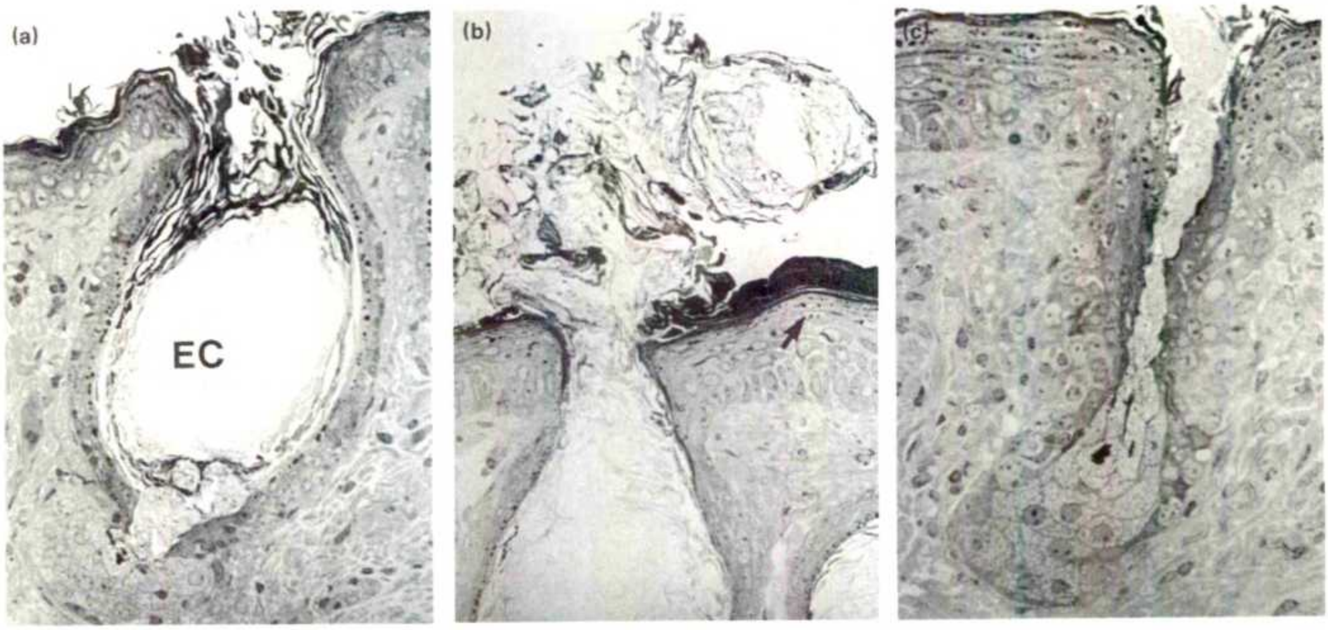

Figure 1. Comedolysis during treatment with retinoic acid. (a) Non-treated rhino mouse skin. EC, epidermal comedo. (b) After 5 days of treatment the epidermis shows a hyperplastic response and an increased granular layer (arrow). (c) After 17 days of treatment there is comedolysis with a decrease in the size and diameter of the comedo. There is no further accumulation of horny material in the follicular canal (× 320).

noted in the epidermis and epithelial wall of the comedones (Fig. 1b) associated with an increased number of epidermal granular cell layers.

Electron microscopy

The earliest ultrastructural changes were observed in the dermis after 2–3 days of treatment. From day 2 to day 5 the mast cells showed evidence of degranulation (Fig. 2), but by day 8 continuing degranulation of these cells was not observed.

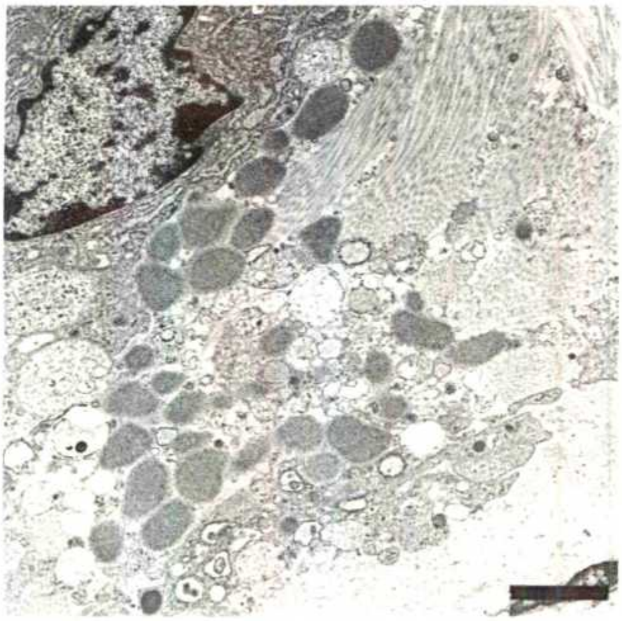

Figure 2. Rhino mouse skin after 2 days of treatment with retinoic acid (0·025%) showing degranulated mast cell in the dermis (bar 2 μm).

Dermal Langerhans cells with typical Birbeck granules, which were numerous in the non-treated rhino mouse skin as well as in the acetone-treated skin, were rapidly affected by the topical treatment with the retinoid. After 3 days of application some clumping of these cells was observed (Fig. 3) and close contact between lymphocytes and Langerhans cells was observed both in the dermis (Fig. 3) and epidermis. Some Langerhans cells were also observed projecting through the basement membrane. These features persisted until day 10 of treatment. Cytoplasmic and membrane alterations of dermal and epidermal Langerhans cells were then seen from days 10–19 of treatment. The number of dermal Langerhans cells showed a significant decrease after 3 weeks of treatment (Table 1).

After 5–8 days of treatment, some alterations in the keratinocytes with intracellular vacuolization, detachment of the nuclear envelope and an enlargement of the intercellular space were seen in the epidermis and epithelial wall of the comedones. These changes disappeared by day 12.

After the first week, correlated with the comedolytic effect observed on histology, the ultrastructural changes primarily concerned proliferation and later differentia-

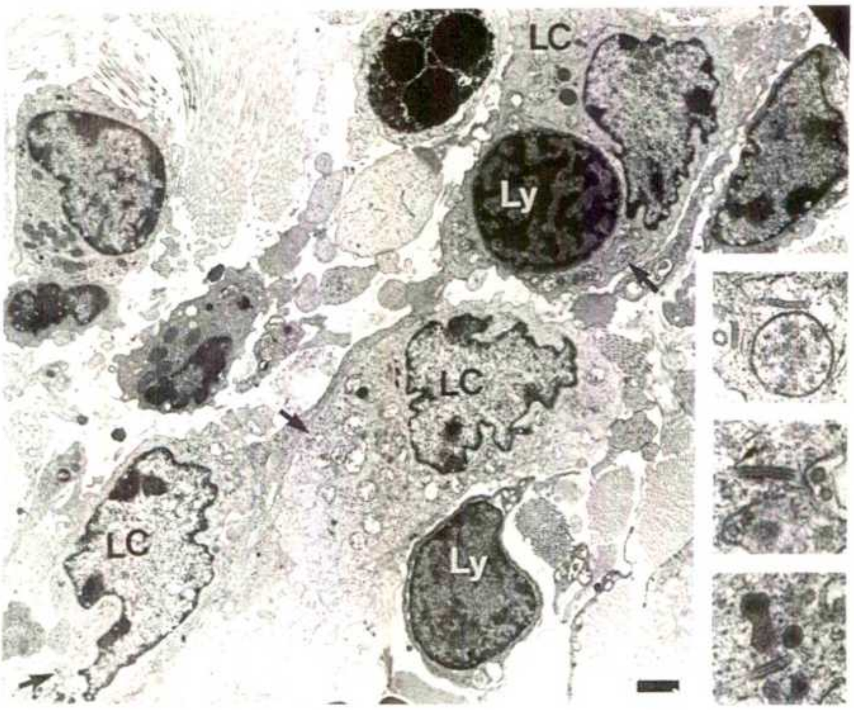

Figure 3. After 3 days of retinoic acid treatment there is close contact between several Langerhans cells (LC) with typical Birbeck granules (arrows and insets) and lymphocytes (Ly) (bar 2 μm).

Table 1. Quantification of dermal Langerhans cells before and after 3 weeks of topical applications of all-*trans* retinoic acid

Treatment	Animal	LC	No. of U.	LC/U	Mean value (±SD)
Non-treated	1	3	8	0·375	
	2	9	25	0·360	
	3	6	18	0·333	
	4	11	21	0·524	
	5	4	12	0·333	
	6	8	16	0·500	
					0·40±0·09
Retinoic-acid-treated mice	7	1	11	0·091	
	8	4	14	0·286	
	9	3	16	0·188	
	10	2	29	0·069	
	11	7	28	0·250	
					0·18±0·10*

$^{*}P<0{\cdot}01$ (Wilcoxon two-sided test); LC, number of Langerhans cells; U, square surface of dermis = 50 μm^2; LC/U, number of Langerhans cells per 50 μm^2 of dermis.

tion of keratinocytes. Hyperplastic features with numerous mitoses were observed. From day 10 to 19, numerous markers of keratinocyte differentiation were modified. The increase in the number of epidermal granular cell layers (Fig. 4), was associated with increased size and number of the keratohyalin granules, which became compact and homogeneously dense. The number of intra- and extracellular membrane coating granules (MCG) was increased both in the epidermis and the follicular epithelium (Fig. 5). Large quantities of extracellular multilamellar material were also observed in the intercellular space of the stratum corneum (Fig. 5b). Using SEM, the shape and surface of the corneocytes were seen to be changed. Numerous features of premature desquamation were observed, including total detachment of the stratum corneum (Fig. 4b), mainly in the infundibular zone of the epithelium wall of comedones.

Mast-cell quantification

The avidin-FITC labelling of mast cells is shown in Figure 6. From day 2 until day 5, the number of these cells decreased but by day 8 the number of mast cells had returned to the levels found in acetone-treated skin. During the third week of treatment, the number of mast cells was greatly increased (Fig. 7).

Discussion

The present study demonstrates that topical applications of all-*trans* retinoic acid on rhino mouse skin induces a dermal response in the early stages which is followed by epidermal and follicular effects that lead to comedolysis.

The early dermal response involves at least three cell types: mast cells, Langerhans cells and lymphocytes. Mast cells appear to be the first cells to respond, with an almost total degranulation after 2 days of treatment. The decrease in the number of mast cells during the same

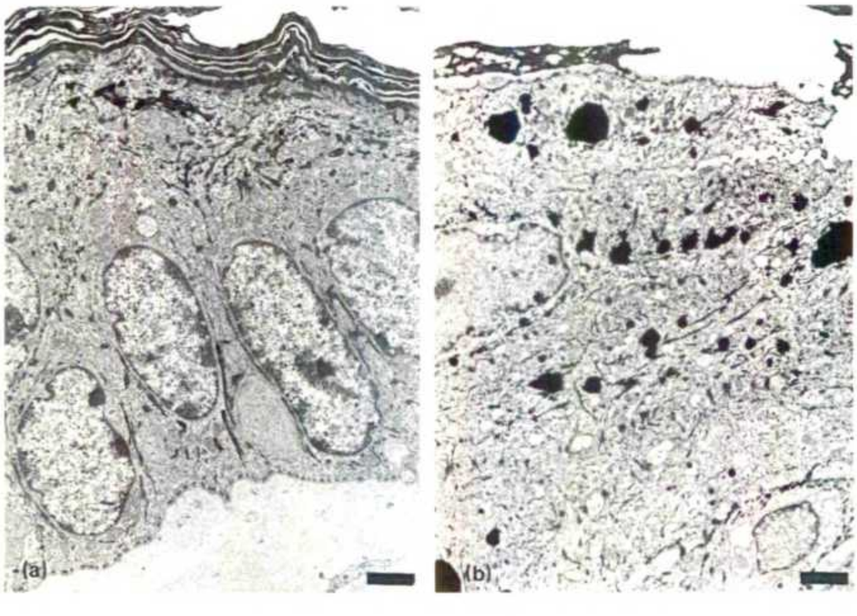

Figure 4. Modifications of the epidermal granular layer during treatment with retinoic acid. (a) Non-treated skin. The epidermal granular layer is composed of two cellular layers. The keratohyalin granules are small and irregular in shape. (b) After 3 weeks of treatment, the granular layer is increased. The keratohyalin granules are large in size and have a regular shape and a dense structure (bars 2 μm).

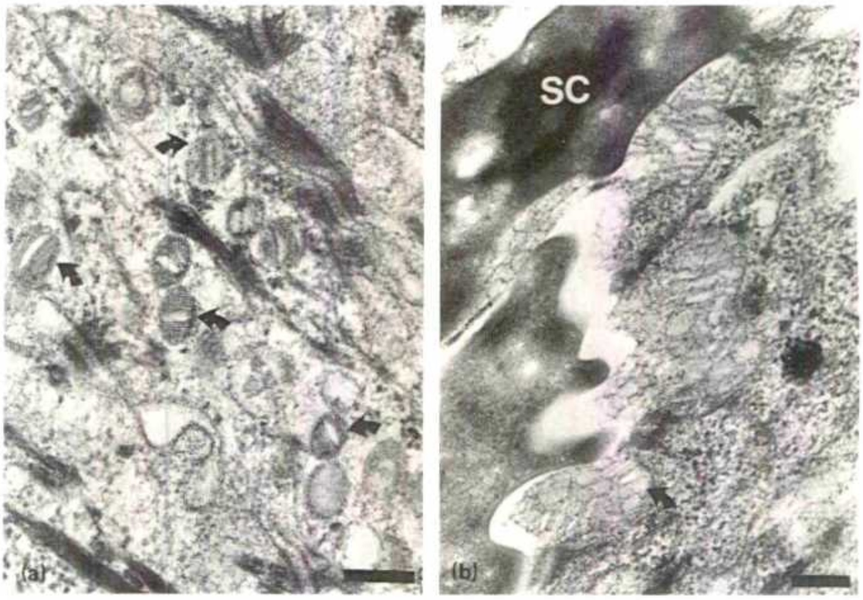

Figure 5. MCG (arrows) in rhino mouse skin treated with retinoic acid. (a) Intracellular MCG. (b) Extracellular MCG and intercellular material in the stratum corneum (SC). (bars 200 nm).

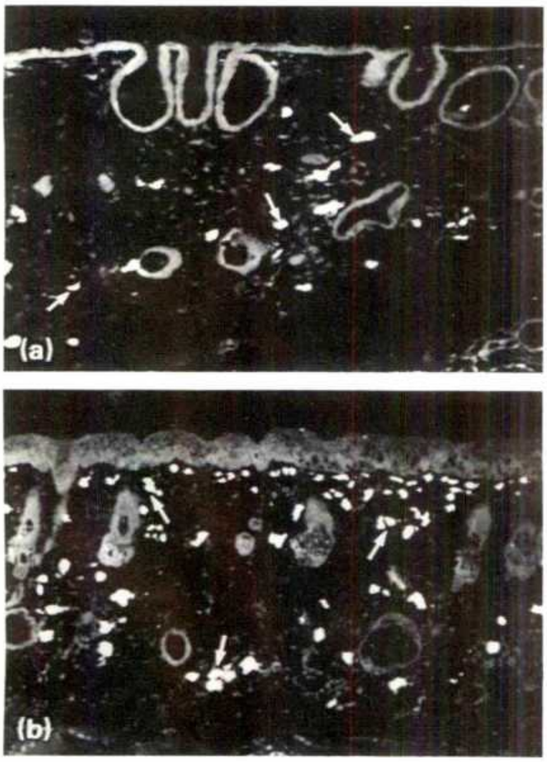

Figure 6. Labelling of mast cells (arrows) in rhino mouse skin by the avidin-FITC method. (a) Non-treated skin. (b) Skin treated for 3 weeks with retinoic acid. The number of mast cells is greatly increased (× 125).

period could be related to this degranulation, because the method used identifies the granules of the cells. A similar degranulation was detected during oral treatment with etretinate[13] and *in-vitro* experiments have

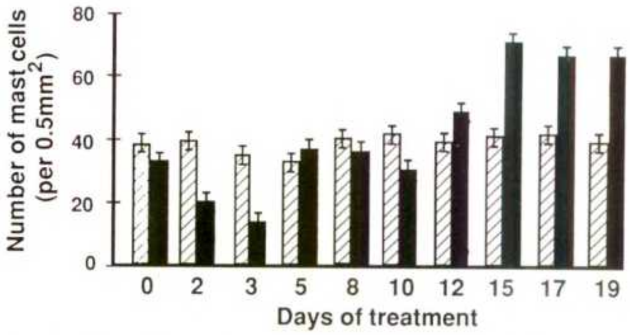

Figure 7. Quantification of the number of mast cells in the dermis during treatment with retinoic acid ■. There is a decreased number at the beginning of the treatment. Similar values to those of acetone-treated skin □ are observed during the second week of treatment, and an increase in the number of mast cells is observed during the third week. Error bars: SEM.

shown that retinoids are able to induce histamine release.[14] During the second week of treatment, the mast-cell number returned to normal and increased strikingly during the third week. A previous *in-vivo* study in colorectal tissue showed similar results.[15]

Dermal Langerhans cells are frequently observed in rhino mouse skin. The unusually high number of this cell type, which is involved in presentation of antigens to T lymphocytes,[16] might be related to alterations in the immune system in the rhino mouse.[17] After 3 days of treatment with retinoic acid, associations between Langerhans cells and lymphocytes were seen and some movement was suggested by the Langerhans cells projecting into the basement membrane. In agreement with previously published reports,[18,19] after 3 weeks of treatment the number of Langerhans cells was severely decreased, and many of the dermal Langerhans cells showed an altered morphology. This occurred simultaneously with the epidermal cytotoxic phase and might be related. In certain human pathological conditions, oral etretinate restores the number and distribution of epidermal Langerhans cells.[20,21] Dermal Langerhans cells have been stimulated by etretinate in psoriasis.[13]

Vitamin A has been previously shown to induce various changes in the immune system,[22] and the involvement of mastocytes, Langerhans cells and lymphocytes strongly suggest that the early dermal response to retinoic acid treatment is mediated via an immune reaction.

The epidermal and epithelial reaction which resulted in comedolysis occurred after 5–8 days, reached a maximum after day 12 and then stabilized. Comedolysis has been shown to be associated with modifications in the proliferation and differentiation of keratinocytes.[4] The hyperproliferative response[23] does not seem to be sufficient to provoke comedolysis,[2,4] and modification of keratinocyte differentiation seems to be necessary.[4] These changes occurred mainly in the upper part of the epidermis and follicular epithelium, leading to an increased desquamation with a disorganized horny layer. This probably caused the expulsion of the follicular plugs. Detailed structural changes of the stratum corneum induced by retinoid therapy have been reported.[5] These changes were also observed in acneic lesions[24] and in animals with experimentally induced comedones.[25] Retinoids have also been shown to modify *in vitro* terminal differentiation of keratinocytes[8] as shown by changes in transglutaminase expression[26] and in cornified envelope formation. All these effects on terminal differentiation and consequently on desquamative function could explain the benefit of such molecules in the treatment of acne.

Acknowledgments

The authors thank Drs C.Hensby, M.Bouclier and A.Chatelus for help and advice and A.Jomard and his colleagues and C.Delain for animal procedures.

References

1 Mann J. Hair loss and cyst formation in hairless and rhino mutant mice. *Anat Rec* 1971; **270:** 465–500.

2 Kligman L, Kligman A. The effect on rhino mouse skin of agents which influence keratinization and exfoliation. *J Invest Dermatol* 1979; **73:** 354–8.

3 Ashton R, Connor M, Lowe N. Histologic changes in the skin of the rhino mouse ($hr^{rh}hr^{rh}$) induced by retinoids. *J Invest Dermatol* 1984; **82:** 632–5.

4 Bernerd F, Ortonne JP, Bouclier M *et al.* The rhino mouse model: the effects of topically applied all-trans retinoic acid and CD 271 on the fine structure of the epidermis and utricle wall of pseudocomedones. *Arch Dermatol Res* 1991; **283:** 100–7.

5 Elias PM. Epidermal effects of retinoids: supramolecular observations and clinical implications. *J Am Acad Dermatol* 1986; **15:** 797–809.

6 Schultz-Ehrenberg U, Orfanos CE. Light and electron microscopic changes under oral retinoid treatment. In: *Retinoids* (Orfanos CE *et al.*, eds). Berlin: Springer-Verlag, 1981; 85-92.

7 Marcelo, C, Madison K. Regulation of the expression of epidermal keratinocyte proliferation and differentiation by vitamin A analogs. *Arch Dermatol Res* 1984; **276:** 381–9.

8 Fuchs E, Green H. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell* 1981; **25:** 251–75.

9 Kligman L. Effects of all-trans-retinoic acid on the dermis of hairless mice. *J Am Acad Dermatol* 1986; **15:** 779–85.

10 Zelickson A, Mottaz J, Weiss M *et al.* Topical tretinoin in photoaging: an ultrastructural study. *J Cutaneous Aging Cosmet Dermatol* 1988; **1:** 41–7.

11 Tsambaos D, Orfanos CE. Effects of oral retinoid on dermal components in human and animal skin. In: *Retinoids* (Orfanos CE *et al.*, eds.) Berlin: Springer-Verlag, 1981; 99–108.

12 Bergstresser P, Tigelaar R, Tharp M. Conjugated avidin identifies cutaneous rodent and human mast cells. *J Invest Dermatol* 1984; **83:** 214–8.

13 Tsambaos D, Orfanos CE. Ultrastructural evidence suggesting an immunomodulatory activity of oral retinoid. Its effect on dermal components in psoriasis. *Br J Dermatol* 1981; **104:** 37–45.

14 Cantwell ME, Foreman JC. The actions of retinal and retinoic acid on histamine release from rat peritoneal mast cells. *Eur J Phamacol* 1989; **160:** 43–51.

15 Brevard PB, Anderson LG, Magee AC. *In-vivo* effects of retinoids on the histological changes in colorectal tissue. *Nutr Rep Int* 1985; **31:** 635–48.

16 Silberberg I. Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. *Acta Derm Venereol (Stockh)* 1973; **53:** 1–12.

17 Kawaji H, Tsukuda R, Nakaguchi T. Immunopathology of rhino mouse, an autosomal recessive mutant with murine lupus-like disease. *Acta Pathol JPN* 1980; **30:** 515–30.

18 Hachisuka H, Uno H. Effects of retinoic acid on the epidermal Langerhans cells and B-glucuronidase activity in macaque skin. *Am J Dermatopathol* 1987; **9:** 316–23.

19 Shiohara T, Kobayashi M, Narimatsu H, Nagashima M. Effect of orally administrated aromatic retinoid on murine Langerhans cells. *Arch Dermatol Res* 1987; **279:** 198–203.

20 Van der Schroeff J, Ruiter D, Bots G. Epidermal Langerhans cells in erythrokeratodermia variabilis. *Arch Dermatol Res* 1982; **274:** 339–48.

21 Haftek M, Faure M, Schmitt D, Thivolet J. Langerhans cells in skin from patients with psoriasis. Quantitative and qualitative study of T6 and HLA-DR antigen expressing cells and changes with aromatic retinoid administration. *J Invest Dermatol* 1983; **81:** 10–14.

22 Dennert G. Retinoids and the immune system: immunostimulation by vitamin A. In: *Retinoids* (Sporn S.M. ed). Vol 2. New York: Academic Press, 1984; 373–90.

23 Connor M, Lowe N. A comparative study of the induction of epidermal hyperplasia by natural and synthetic retinoids. *J Pharmacol Exp Ther* 1986; **237:** 31–5.

24 Wolff H, Plewig G, Braun-Falco O. Ultrastructure of human sebaceous follicles and comedones following treatment with vitamin A acid. *Acta Derm Venereol (Stockh)* 1975: **Suppl. 74:** 99–110.

25 Woo-Sam PC. The effect of vitamin A acid on experimentally induced comedones: an electron microscopic study. *Br J Dermatol* 1979: **100:** 267–76.

26 Rubin A, Rice R. Differential regulation by retinoic acid and calcium of transglutaminase in cultured neoplastic and normal human keratinocytes. *Cancer Res* 1986; **46:** 2356–61.

British Journal of Dermatology (1991) 125, 426–435.

Cell adhesion in Hailey–Hailey disease and Darier's disease: immunocytological and explant-tissue-culture studies

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Accepted for publication 5 June 1991

Summary

The pathogenesis of Hailey–Hailey disease and Darier's disease was investigated using immunocytological and explant-tissue-culture techniques. There was breakdown of the intercellular adhesions between keratinocytes in explants from clinically uninvolved skin of patients with Hailey–Hailey disease or Darier's disease. The major desmosomal components were present in the cultures and were expressed in a punctate peripheral pattern at cell–cell contact sites, but there was diffuse staining of acantholytic cells. Plasminogen, which is expressed by basal keratinocytes in normal skin, was detected in association with suprabasal acantholytic cells in skin biopsies from these diseases. Plasminogen was reversibly displaced from the cells by 6-aminohexanoic acid, suggesting that binding is mediated by a reaction with the lysine receptor on the plasminogen molecule. Plasminogen was also detected in separating cells in explant cultures and there was cytoplasmic expression of the plasminogen activator urokinase by these cells.

These abnormalities are not unique to either disease and do not account for the phenotypic differences between Darier's disease and Hailey–Hailey disease, but plasmin generation may have a role in perpetuating cell separation.

Hailey–Hailey disease (benign familial chronic pemphigus) and Darier's disease (keratosis follicularis) are both dominantly inherited skin diseases in which there is abnormal epidermal cell adhesion.[1,2] Darier's disease is characterized by the presence of itchy keratotic papules predominantly on the upper trunk and scalp, while Hailey–Hailey disease presents with uncomfortable erosions or eczematous, crusted lesions in flexures or at sites of trauma. Blisters may occur in both diseases but skin fragility is more marked in Hailey–Hailey disease. Suction causes intraepidermal cleavage in clinically uninvolved skin in Hailey–Hailey disease[3] but suction always causes subepidermal cleavage in normal individuals and in clinically uninvolved skin in Darier's disease.[4] There is considerable histological overlap between the diseases and in both there is breakdown of the desmosome–keratin filament complexes with subsequent loss of cohesion between epidermal cells (acantholysis).[5–10] Immunocytological studies of skin biopsies have demonstrated that the major desmosomal components are present within the epidermis in both diseases,[11] but these results do not exclude the possibility that there is a structural abnormality in a desmosomal component or that there may be an abnormality in the regulation of desmosome formation or breakdown. The pathogenesis of both diseases remains uncertain.

The mechanisms which control the formation of adhesive junctions at cell–cell contact sites are complex and incompletely understood. Appropriate adhesion is required for normal growth, mitosis or differentiation. Plasminogen activators, which influence epidermal cell division, differentiation and migration[12–17] may also influence adhesion.[18] Plasminogen is detected in the basal layer of the epidermis,[19,20] plasmin splits desmosomes *in vitro*[21,22] and plasminogen activation has been implicated in the pathogenesis of acantholysis in the autoimmune disease pemphigus.[18] Plasminogen is expressed by the acantholytic cells within lesional skin in Darier's disease.[20] Comparative immunocytological studies have not been performed in Hailey–Hailey disease, but plasminogen activator activity is increased in lesional skin in Hailey–Hailey disease.[15,23] Plasminogen activation may contribute to acantholysis in both diseases.

The present study was designed to compare the immunocytological characteristics and keratinocyte ad-

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hesion in Darier's disease and Hailey–Hailey disease. Skin biopsies from patients with Hailey–Hailey disease were examined by indirect immunofluorescence with antibodies to plasminogen and plasminogen activators for comparison with previous observations in Darier's disease.[20] The characteristics of plasminogen binding sites in the epidermis in Darier's disease and Hailey–Hailey disease were explored by treating sections with 6-aminohexanoic acid (6AHA) which interacts specifically with lysine-binding sites on the plasminogen molecule to displace plasminogen from its substrate.[24] The acantholytic process was examined by establishing explants of skin from patients with Hailey–Hailey disease or Darier's disease in culture. The pattern of growth around the explants was recorded and the *in-vitro* expression of the major desmosomal components, plasminogen and the plasminogen activators, tissue plasminogen activator (tPA) and urokinase (uPA), was studied.

Methods

Tissue

Biopsies were taken from five patients with Hailey–Hailey disease and two patients with Darier's disease, snap frozen and stored in liquid nitrogen until frozen sections were cut. The biopsies included involved and perilesional skin. Samples of normal skin obtained from surgical specimens were used as controls.

Approval for the study was obtained from the Central Oxford Research Ethical Committee and informed consent was obtained from the patients.

Chemicals

6AHA was obtained from Sigma Chemical Company, Poole, U.K. Lys-plasminogen was prepared from a modified Cohn Fraction III supplied as a frozen paste from the National Blood Transfusion Centre, Elstree, U.K., using Sepharose-bound lysine essentially as previously described.[25] The displaced plasminogen was gel filtered and lyophilized in 10-mg quantities. The plasminogen showed a typical doublet when analysed by SDS gel electrophoresis. Lys-plasminogen was dissolved in phosphate-buffered saline prior to use at a final concentration of 10 μg/ml determined by using an absorption coefficient ($E_{280\,\text{nm}}$ 1%) of 16.

Plasminogen receptor studies

Endogenous plasminogen was displaced from the epidermis by incubating skin sections in 0·1 mol/l 6AHA for 30 min followed by thorough washing in 3 × 200 ml 0·05 mol/l phosphate, NaCl 0·15 mol/l buffer, pH 7·4 (PBS) for 30 min. Plasminogen was replaced by incubating sections for 60 min at room temperature with 10 μg/ml lys-plasminogen. Plasminogen distribution was assessed immunohistologically.

Explant tissue culture

Superficial 4-mm punch biopsies were obtained under local anaesthesia. Biopsies were taken from clinically normal and involved skin on the trunk of 10 patients with histologically confirmed Darier's disease and from clinically normal skin on the trunk of six patients with histologically confirmed Hailey–Hailey disease (Tables 1 and 2). Cultures were not prepared from the involved skin in Hailey–Hailey disease because the lesions were often secondarily infected and the acantholytic epidermis was fragile and difficult to handle. Normal skin was obtained from the trunk of 10 patients during routine surgical procedures. The skin biopsies were placed in Dulbecco's modified Eagle's medium (DMEM) and prepared for culture within 3 h.

Skin was prepared for culture under sterile conditions. The biopsy was dipped briefly in 70% ethanol and washed in DMEM. After removing any subcutaneous tissue, the biopsy was cut into 1–2-mm-diameter fragments using a scalpel. The fragments were placed on a 50-mm-diameter culture dish (Cell-cult tissue culture products, Sterilin Ltd, Hounslow, U.K.) orientated so that the dermis was in contact with the base of the dish and allowed to dry for 2–3 min to promote adherence before adding 5 ml of culture medium. Explant cultures of normal skin were set up on the same day and under the same conditions as cultures from patients with Darier's disease or Hailey–Hailey disease. Cultures were maintained in DMEM buffered with 20 mM HEPES (4·766 g/l) and supplemented with 10% newborn bovine serum (NBS), glutamine (584·0 mg/l), sodium bicarbonate (2 g/l), penicillin (5000 IU/ml) and streptomycin (5000 μg/ml) and incubated at 37°C. All the culture reagents were obtained from Flow Lab Ltd, Irvine, U.K. The cultures were maintained for a minimum of 5 to a maximum of 42 days. Cell outgrowths were observed and photographed using an Olympus inverted microscope (model CK, Olympus Optical Company Ltd, Tokyo) with a phase-contrast attachment.

The explants were prepared for immunocytology by placing the culture dishes on ice, washing thoroughly with cold PBS and flooding the dishes with 50%

Table 1. Darier's disease

Sex	Age	Treatment	Clinically uninvolved skin: Explants established	Clinically uninvolved skin: Explants showing abnormal growth	Involved skin: Explants established	Involved skin: Explants showing abnormal growth
M	24	Etretinate occasionally	2	0		
M	37	None	5	5	14	11
F	41	None	7	0	8	4
M	59	None	6	4	7	2
F	27	None			3	0
M	66	Etretinate	3	0	5	2
F	43	None	9	5	6	6
M	19	None	4	0	3	0
M	28	None	5	0	2	0
M	30	None	5	0	6	0
Total			46	14 (30%)	54	25 (46%)

Table 2. Hailey–Hailey disease

Sex	Age	Clinically uninvolved skin: Explants established	Clinically uninvolved skin: Explants showing abnormal growth
F	74	5	5
M	39	4	4
F	65	6	6
M	68	5	5
M	54	5	0
F	18	4	2
Total		29	22 (76%)

methanol/50% acetone for 2 min. After further washing in PBS the dishes were air-dried and stored at −70°C.

Antibodies

Polyclonal antibodies against tissue plasminogen activator (tPA) (W50), urokinase (uPA) (W44), and plasminogen (W05) were raised in rabbits and characterized in the Oxford Fibrinolysis Research laboratory as previously described.[20] Antibodies against tPA, uPA and plasminogen were used at a dilution of 1/800 in PBS with 10% normal swine serum (NSS). Normal rabbit serum (NRS) diluted 1/800 in PBS with 10% NSS was used as a control.

Mouse monoclonal antibodies to desmosomal proteins 1 and 2 (dp1/2; desmoplakins); desmosomal glycoprotein 1 (dg1; desmoglein) and desmosomal glycoprotein 2 and 3 (dg2/3; desmocollins) were raised in the Cancer Research Campaign Medical Oncology Unit, Southampton.[11] Optimum working dilutions were 1 : 10 in PBS for anti-dp1/2 and neat for anti-dg1 and anti-dg2/3.

Immunocytology

Frozen sections. Unfixed 8-μm frozen sections were placed on multiwelled slides, air-dried and incubated with the primary antibody for 60 min at room temperature.

Cells. The dishes were defrosted and air-dried. The fragments of tissue were carefully removed leaving the explant outgrowths on the dishes. Each explant was isolated from its neighbours by encircling it with water-repellent material using a Dako pen (Dako Ltd, Copenhagen, Denmark). Explants were incubated with the primary antibody, NRS or PBS for 60 min at room temperature (Table 3).

After washing, specimens were either incubated with swine anti-rabbit or rabbit anti-mouse immunoglobulin peroxidase-labelled conjugate (Dako Ltd, Copenhagen) diluted 1/50 in PBS with 10% FCS and 3% human sera or fluorescein-conjugated swine anti-rabbit or rabbit anti-mouse immunoglobulin (Dako Ltd, Copenhagen) used at a dilution of 1/50 in PBS. Incubations were carried out for 60 min at room temperature and specimens were given 3 × 10 min washes in PBS. Fluorescein-labelled specimens were mounted in Citifluor (Glycerol/PBS solution, Agar Aids, Stanstead, Essex,

Table 3. Number of explants stained with each antibody

	Anti-plasminogen (W05)	Anti-tPA (W50)	Anti-uPA (W44)	Anti-dp (11-5F)	Anti dg (33.3D)	(52.3D)
Explants						
Normal skin	6	7	7	3	4	6
Darier's disease						
Uninvolved	8	6	6	3	3	3
Involved	8	6	9	8	5	6
Hailey–Hailey disease						
Uninvolved	4	4	7	6	1	1

U.K.). The colour was developed in peroxidase-labelled specimens using diaminobenzidene and then specimens were mounted in Citifluor. The sides were cut off the culture dishes. Specimens were examined and photographed either by light microscopy or by epifluorescence using an Olympus Photomicroscope (model BHS with BH2 RFL attachment).

Results

Morphology of explant cultures

Normal skin. Sixty explants of normal skin were established in culture. A cohesive outgrowth of keratinocytes was visible around the edges of the explants after 48 h. Over the following 2 weeks the outgrowth gradually expanded to form a flat, well organized keratinizing sheet (Fig. 1). The cells were uniform in size and those at the growing edge remained in contact with their neighbours. In the more mature central areas near the explant there was stratification with shedding of cells from the surface of the sheet.

Darier's disease. Forty-six explants of clinically uninvolved skin from 10 patients and 54 explants of involved skin from nine patients were established in culture (Table 1). A fringe of growth was visible around the explants after 48–72 h. The pattern of growth was abnormal in 30% of the explants obtained from clinically uninvolved skin and 46% of the explants derived from involved skin. There was rounding up and separation of cells at the advancing edge of the sheet of cells (Fig. 2a) and separation and irregularity within the expanding sheet in 14/20 of the uninvolved skin explants from three patients and in 25/40 of the involved skin explants from five patients.

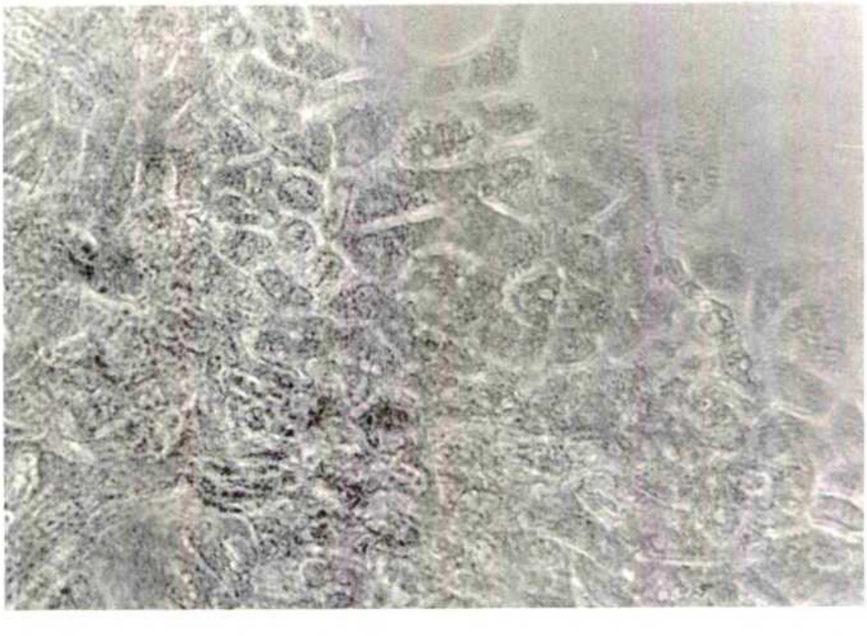

Figure 1. Explant of normal skin. The cohesive outgrowth of keratinocytes forms a well-organized sheet which stratifies as the cells mature (phase-contrast light microscopy).

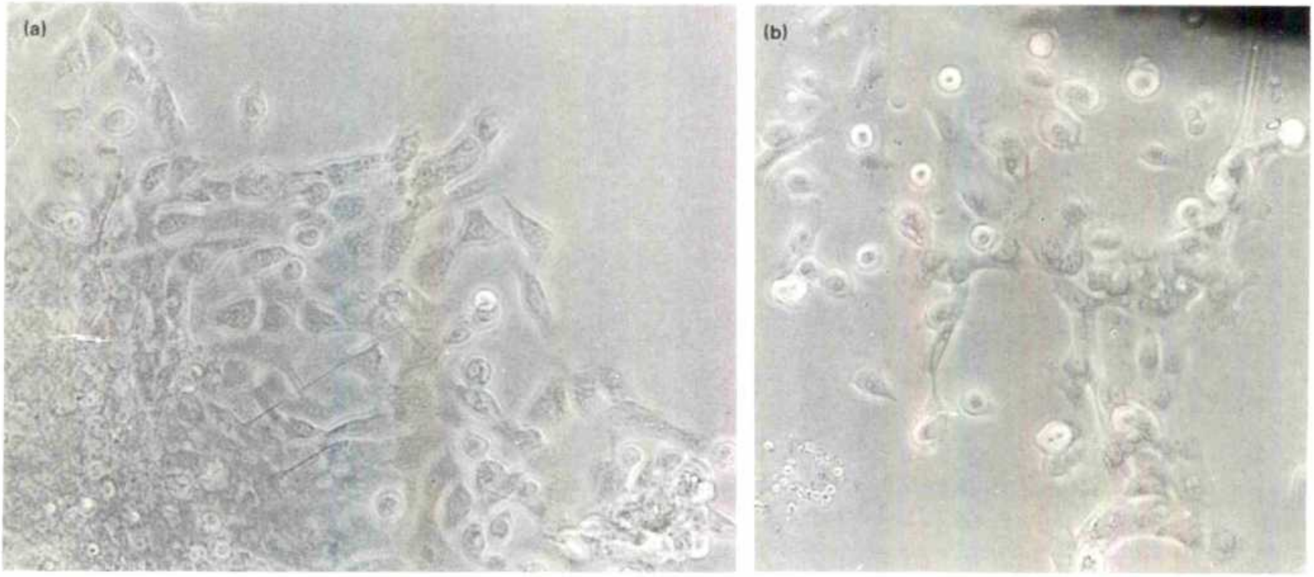

Figure 2. Explants of (a) clinically normal skin from Darier's disease and (b) clinically normal skin from Hailey–Hailey disease. The outgrowth of keratinocytes is discohesive and disorganized. Some of the cells are rounded up and have no intercelluar contacts while others are connected by attenuated cytoplasmic strands (phase-contrast light microscopy).

Hailey–Hailey disease. Twenty-nine explants of clinically uninvolved skin from six patients were established in culture (Table 2). A fringe of growth around the explant was visible after 48–72 h and this extended over subsequent days. In five of the six patients the pattern of growth was abnormal in 22/24 of the explants. There was loss of cohesion in the sheet of keratinocytes. Individual cells at the advancing edge rounded up and separated from adjacent cells. Some of the cells maintained contact with their neighbours by means of long thin cytoplasmic extensions (Fig. 2b). The pattern of growth was abnormal in 76% of the explants.

Desmosomal components in explants

Dp 1/2. In normal skin there was punctate staining concentrated at the periphery of the cells where they were in contact with adjacent cells, but there was no staining at the free margin of the cells at the advancing edge of the culture. In areas of stratification where desmosomes connected stratifying cells with underlying basal cells there was also punctate staining over the surface of the cells and this was often in a linear pattern. There was normal punctate staining at the periphery of the cells in areas of cell–cell contact in the explants from Darier's disease and Hailey–Hailey disease, but in the dissociating cells this peripheral punctate stain was replaced by strong diffuse cytoplasmic staining (Fig. 3). Sometimes stain was visible within the attenuated cytoplasmic projections.

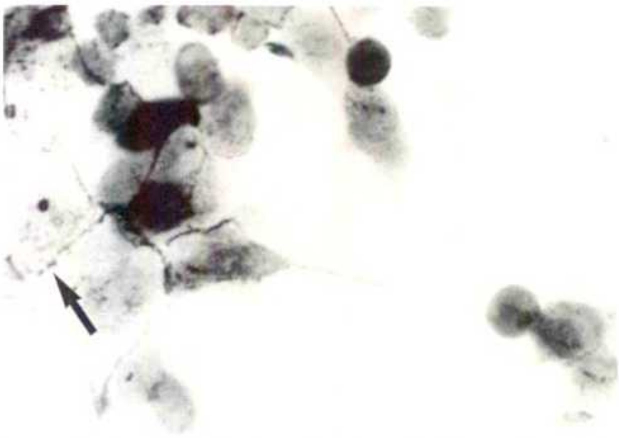

Figure 3. Explant of clinically uninvolved skin from Hailey–Hailey disease stained by indirect immunoperoxidase with antibodies to dp1/2 and weakly counterstained with haematoxylin. Punctate peripheral staining is visible at cell–cell contact sites in the cohesive areas of the outgrowth [→] but there is diffuse staining of the rounded up acantholytic cells.

Dg1 and dg2/3. In normal skin the expression of dgs was weak at the periphery of cells at the growing edge of the explants and stronger in the differentiating cells. In Darier's disease and Hailey–Hailey disease the findings were similar to those found with the dps. There was weak peripheral expression of dgs in the cohesive areas but there was weak diffuse staining of dissociating cells.

Protease studies: skin biopsies

Plasminogen. The results in Hailey–Hailey disease were similar to those previously obtained in Darier's disease.[20] Plasminogen was detected in a normal pattern in association with the basal cells in perilesional skin, but in lesional skin plasminogen was also detected in association with the suprabasal acantholytic cells (Fig. 4a).

Plasminogen receptor studies. Sections from Darier's disease (two) or Hailey–Hailey disease (two) were incubated with 6AHA. Plasminogen was displaced from the majority of the acantholytic keratinocytes as well as the basal keratinocytes (Fig. 4b). Plasminogen could be restored to the cells by incubating the sections with lysyl plasminogen 10 μg/ml for 30 min (Fig. 4c). We suggest that plasminogen binds to the surface of cells via a reversible interaction involving the lysine receptor. Some residual plasminogen was detected in the keratotic plug in the lesions of Darier's disease.

uPA and tPA. These were not detected in normal or involved epidermis using indirect immunofluorescence.

Protease distribution in explants

Plasminogen. There was weak staining of cohesive cells at the growing edge of the cultures, diffuse staining of keratinocytes throughout the rest of the cultures and in areas of stratification and strong expression in cells which were rounding up and being shed from the surface of the sheet. There was also intense diffuse staining of the dissociating, rounded-up cells at the advancing edge of the explants from Darier's disease and Hailey–Hailey disease (Fig. 5a).

tPA. tPA was not detected in the explant cultures (Fig. 5b).

uPA. uPA was detected in an irregular granular pattern throughout all the cultures but unlike plasminogen, uPA was also detected in association with the cell membrane at the advancing free margin of the culture. In areas of stratification, uPA was expressed by cells which were being shed from the surface of the cultures. In the explants from Darier's disease and Hailey–Hailey disease uPA was also detected within the dissociating cells at the advancing edge of the culture. There were dense clumps of perinuclear staining within these cells (Fig. 5c).

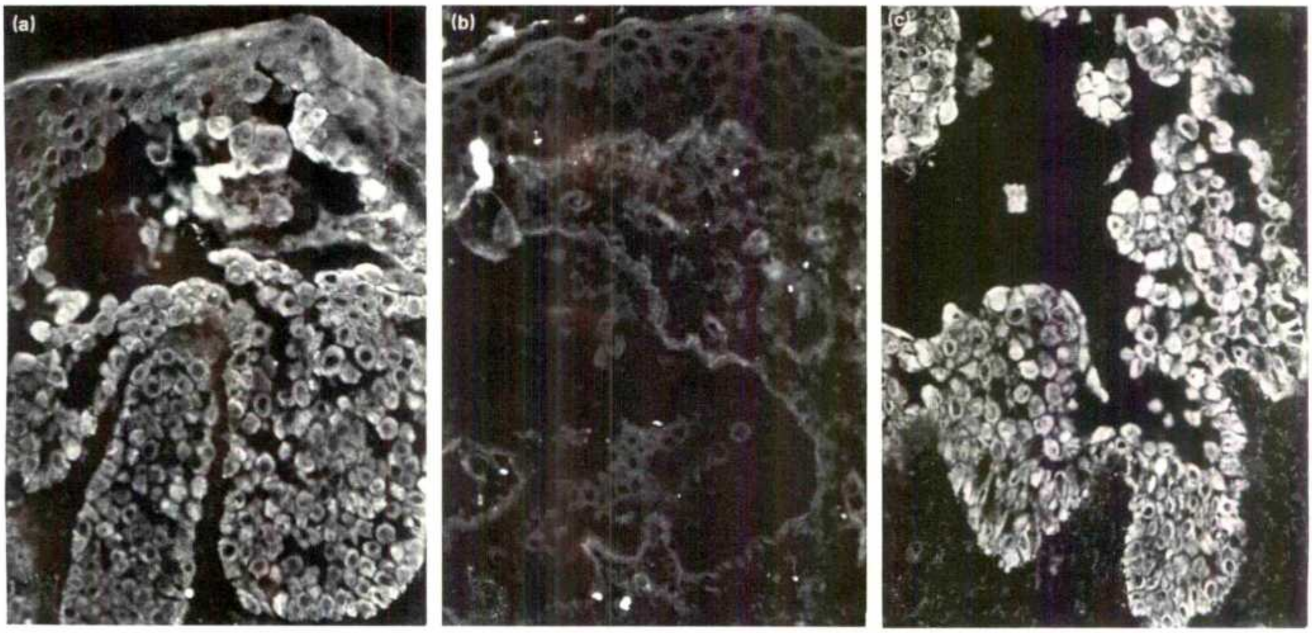

Figure 4. Lesional skin in Hailey–Hailey disease stained by indirect immunofluorescence with an antibody to plasminogen (W05). (a) Fluorescence in the acantholytic cells throughout the lesion. (b) The skin section was treated with 0·1 mol/l 6AHA and plasminogen was displaced from the cells. (c) Plasminogen could be restored to the cells by incubating the section with lysyl plasminogen 10 μg/ml for 30 min.

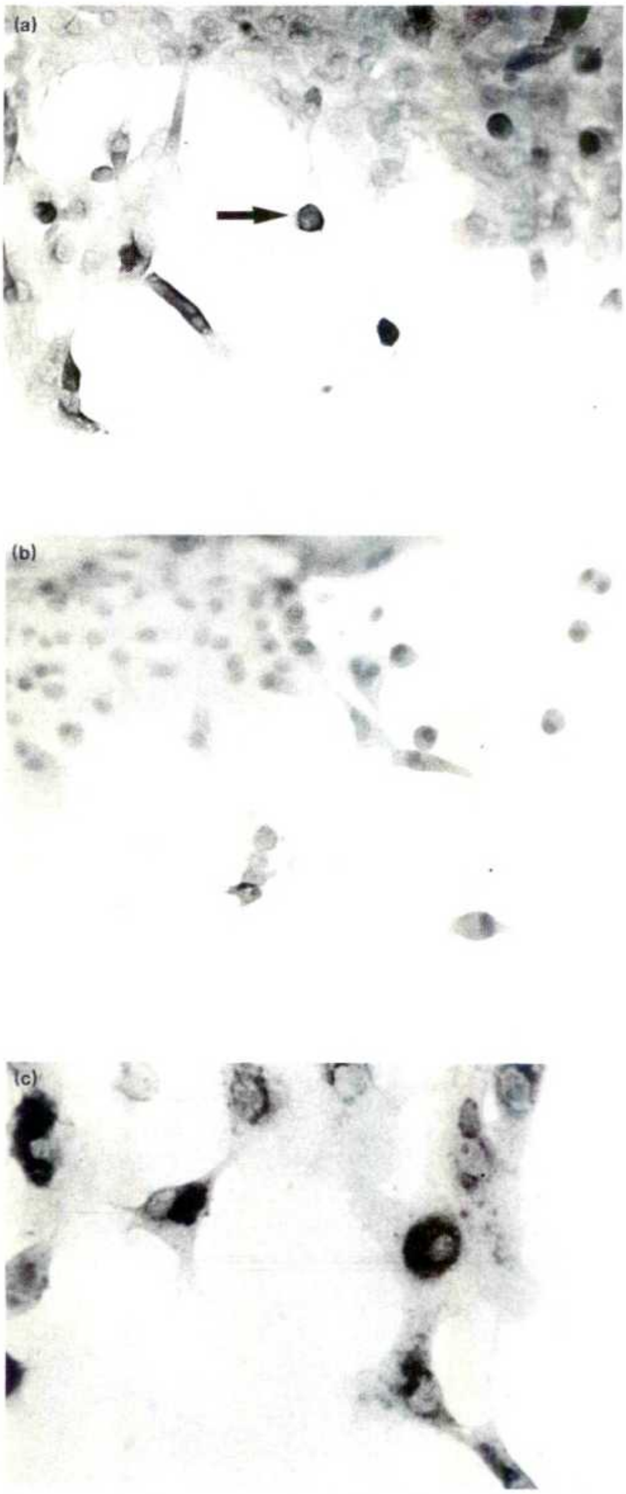

Figure 5. Explant of clinically uninvolved skin from Hailey–Hailey disease stained by indirect immunoperoxidase with antibodies to (a) plasminogen (W05), (b) tPA (W50) or (c) uPA (W44) and weakly counterstained with haematoxylin. (a) The separating keratinocytes are stained diffusely for plasminogen [→]. (b) tPA is not detected in the explant. (c) uPA is detected in a perinuclear distribution within the acantholytic keratinocytes.

Discussion

This study has demonstrated abnormalities in the adhesive behaviour of explanted keratinocytes and changes in the distribution of staining for components of the plasminogen–plasmin system, desmosomal proteins and desmosomal glycoproteins in acantholytic cells in Darier's disease and Hailey–Hailey disease. The findings were similar in the two diseases and none of the results suggest an explanation for the differences between the diseases.

Cell dissociation was observed in explant cultures derived from the clinically normal skin of patients with Darier's disease or Hailey–Hailey disease. Abnormal adhesion was more marked in the cultures of cells from patients with Hailey–Hailey disease than in those from patients with Darier's disease but there were some areas of normal cohesive growth in each explant and in some explants the entire outgrowth was cohesive. Similar abnormal patterns of growth have been observed in explants from Darier's disease and Hailey–Hailey disease[26,27] and cultured keratinocytes from Hailey–Hailey disease.[28,29] There is a generalized abnormality in keratinocyte adhesion in these diseases but it is only revealed under certain conditions. The triggers for desmosomal junction breakdown have not been defined.

In order to examine the contribution of the major desmosomal components to acantholysis, the explants were stained with antibodies to dp1/2, dg1 and dg2/3. In the normal skin the major desmosomal components were detected in a punctate distribution on the surface of the keratinocytes in areas of cell–cell contact. There was punctate intercellular expression of dps throughout the culture, weak expression of dgs in areas of cell–cell contact at the advancing edge of the culture and stronger expression of dgs by differentiating cells nearer the centre of the explant. The results were similar to those described in previous studies.[30] There was similar peripheral punctate staining around the keratinocytes in the cohesive areas in the explants of skin from Darier's disease and Hailey–Hailey disease, but in areas of cell separation, the acantholytic cells lost the punctate membrane staining and were stained diffusely. Dps were expressed more strongly than dgs. The diffuse patterns of staining which we have demonstrated in the acantholytic cells in culture are similar to those seen within epithelial cells cultured in low-calcium medium.[30–34] These cells lose their cell–cell attachments with internalization and degradation of desmosomes. Diffuse cytoplasmic fluorescence with dp antibodies is also observed when the desmosomes of a rat bladder carcinoma line are destabilized. Biochemical analysis suggests that a considerable portion of the dp pool is soluble.[35] The acantholytic cells in skin biopsies from both diseases also stained diffusely for desmosomal components.[11] The cytoplasmic expression of desmosomal components in the acantholytic cells in Darier's disease and Hailey–Hailey disease may reflect solubilization and proteolytic degradation of internalized desmosomal components. Breakdown of desmosomes may be secondary to a subtle structural abnormality in a desmosomal component or another cell adhesion molecule.

Normal epidermis contains components of the plasminogen–plasmin system which are important growth regulators[12–17] and which may contribute to acantholysis.[18] In order to explore the role of proteases in the acantholytic process, skin biopsies and cultured cells were stained immunocytologically for plasminogen and the plasminogen activators, uPA and tPA. Normally plasminogen is detected in association with the basal keratinocytes but is no longer expressed immunocytologically once the cell moves into the suprabasal compartment.[19,20] This change may be secondary to internalization or blocking of plasminogen receptors on suprabasal cells. Suprabasal cells in Hailey–Hailey disease re-express or continue to express plasminogen when they become acantholytic. There is a similar change in plasminogen expression in the acantholytic cells in the lesions of Darier's disease.[20] Plasminogen is also detected in association with the dissociating cells in explant cultures from both diseases. Plasminogen may be binding to a cell surface receptor on both normal basal cells and acantholytic cells as it is reversibly displaced by 6AHA. Plasminogen receptor expression is rapidly and reversibly upregulated in non-adherent monocytoid cells and enhanced plasminogen binding is correlated with an increased capacity to generate plasmin.[36] Cell-associated plasmin probably plays a physiological role in regulating cell migration in non-adherent cells, but in diseases plasmin generation may interfere with re-attachment of acantholytic cells.

As keratinocytes differentiate, intracellular levels of plasminogen activator (PA) increase so that the highest levels are detected in cells shed from differentiating cultures.[14] We were unable to detect PAs immunocytologically in intact skin, but in culture our results demonstrated that uPA was strongly expressed by differentiated cells being shed from the surface of the epithelial sheets and on the free margin of cells at the leading edge of the normal explant cultures. uPA is detected in differentiating keratinocytes being shed from the surface of neonatal cell cultures[12,15,16] and may have a physiological role in desquamation. uPA has also been demonstrated in keratinocytes at the edge of healing wounds[13] as well as in the migrating edge of colonies of keratinocytes in culture.[12,16] Specific receptors for the A chain of uPA are present on the surface of keratinocytes[17] and interaction of epidermal keratinocytes with uPA stimulates cell movement. This reaction appears to be independent of plasmin generation[17,37] and uPA may have an important role in controlling cell migration.

There was strong focal perinuclear expression of uPA within the dissociating cells at the edges of the cultures of cells from Darier's disease and Hailey–Hailey disease. This pattern of staining was not seen in the cohesive cells at the edge of the normal explants. uPA gene expression with synthesis of the protein is induced in porcine kidney cells both by disruption of cytoskeletal structures and by maintaining cells in suspension.[38] Changes in cell shape have a profound effect on uPA mRNA with secondary alterations in the adhesive properties of the cells.[38] The increased intracellular expression of uPA which we have demonstrated in the acantholytic keratinocytes may be secondary to the loss of cell-surface attachments and change in the shape of the cell.

We did not detect tPA in the explants. This is consistent with the observations that mRNA for tPA is not detected in normal epidermis and tPA has not been detected immunocytologically in sections of normal epidermis in previous studies.[15,23] Previous authors have found both immunocytological and biochemical evidence that cultured keratinocytes contain tPA.[16] The discrepancy between this data and our own immunocytological findings in the explants may reflect variation in antibody specificities or sensitivities but there are other possible explanations. Previous data was generally derived from cultures of neonatal foreskin. Cultured neonatal keratinocytes contain mRNA for tPA as well as uPA and therefore resemble pathological epidermis.[23] We have used explants of adult keratinocytes which may be more similar to normal adult epidermis and the presence of dermal fibroblasts in the explants may modify the behaviour of the cells. The neonatal keratinocytes in previous studies had been exposed to trypsin and passaged which may alter the characteristics of the cells. We did not supplement our culture medium with epidermal growth factor, hydrocortisone or cholera toxin, which were generally used in previous studies. Abnormally high amounts of tPA enzymatic activity have been detected in Hailey–Hailey disease as well as a number of other unrelated conditions, and the authors consider that this reflects a non-specific epidermal response to the disease processes rather than implying that tPA has a pathogenic role in the diseases.[15,23]

We suggest that the changes in expression of plasminogen and uPA which we have demonstrated in the acantholytic cells occur after the breakdown in cell adhesion. Once a cell has lost its surface attachments, it rounds up and there are profound changes in the cytoskeleton. Signals which influence receptor expression may be transmitted to the cell membrane, or messages may pass from the cell membrane into the nucleus to alter protein synthesis. Alterations in morphology may modify the expression of both intracellular and cell surface proteases. The results indicate that the acantholytic cells have proteolytic potential but the role that proteolysis plays in the initiation of acantholysis is uncertain. Plasmin generation may augment acantholysis or prevent reattachment of cells. The abnormalities which we have detected are not unique to either disease and do not account for the phenotypic differences in the diseases.

Acknowledgments

The authors would like to thank the dermatologists who referred patients for the study. S.B. was supported by a British Association of Dermatologists Fellowship and The Skin Disease Research Fund. D.G. is supported by the Cancer Research Campaign. We are grateful to Dr S. Houlbroke for advice.

References

1 Wilkinson JD. Darier's disease. In: *Textbook of Dermatology* (Rook A, Wilkinson DS, Ebling FJG, Champion RH, Burton JL, eds), 4th edn. Oxford, England: Blackwell Scientific Publications, 1986; 1439–42.

2 Pye RJ. Benign familial chronic pemphigus (Hailey–Hailey disease). In: *Textbook of Dermatology* (Rook A, Wilkinson DS, Ebling FJG, Champion RH, Burton JL, eds), 4th edn. Oxford, England: Blackwell Scientific Publications, 1986; 1629–31.

3 Burge SM, Millard PR, Wojnarowska F. Hailey–Hailey disease: a widespread abnormality of cell adhesion. *Br J Dermatol* 1991; **124:** 329–32.

4 Burge SM, Millard PR, Wojnarowska F, Ryan TJ. Darier's disease: a focal abnormality of cell adhesion. *J Cutan Pathol* 1990; **17:** 160–4.

5 Mann PR, Haye KR. An electron microscopic study on the acantholytic and dyskeratotic processes in Darier's disease. *Br J Dermatol* 1970; **82:** 561–6.

6 Wilgram GF, Caulfield JB, Lever WF. An electronmicroscopic study of acantholysis and dyskeratosis in Hailey and Hailey's disease. *J Invest Dermatol* 1962; **39:** 373–81.

7 Gottlieb SK, Lutzner MA. Hailey–Hailey disease: an electron microscopic study. *J Invest Dermatol* 1970; **54:** 368–76.

8 Gottlieb SK, Lutzner MA. Darier's disease. An electron microscopic study. *Arch Dermatol* 1973; **107:** 225–30.

9 Caulfield JB, Wilgram GF. An electron-microscopic study of dyskeratosis and acantholysis in Darier's disease. *J Invest Dermatol* 1963; **41:** 57–65.

10 Ishibashi Y, Kajiwara Y, Andoh I *et al.* The nature and pathogenesis of dyskeratosis in Hailey–Hailey's disease and Darier's disease. *J Dermatol (Tokyo)* 1984; **11:** 335–53.

11 Burge SM, Garrod DR. An immunohistochemical study of desmosomes in Darier's disease and Hailey–Hailey disease. *Br J Dermatol* 1991; **124:** 242–51.

12 Morioka S, Lazarus GS, Baird JL, Jensen PJ. Migrating keratinocytes express urokinase-type plasminogen activator. *J Invest Dermatol* 1987; **88:** 418–23.

13 Grøndahl-Hansen J, Lund LR, Ralfkiær E *et al.* Urokinase- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization *in vivo*. *J Invest Dermatol* 1988; **90:** 790–5.

14 Isseroff RR, Fusenig NE, Rifkin DB. Plasminogen activator in differentiating mouse keratinocytes. *J Invest Dermatol* 1983; **80:** 217–22.

15 Jensen PJ, Baird J, Morioka S *et al.* Epidermal plasminogen activator is abnormal in cutaneous lesions. *J Invest Dermatol* 1988; **90:** 777–82.

16 Jensen PJ, John M, Baird J. Urokinase and tissue type plasminogen activators in human keratinocyte culture. *Exp Cell Res* 1990; **187:** 162–9.

17 Del Rosso M, Fibbi G, Dini G *et al.* Role of specific membrane receptors in urokinase-dependent migration of human keratinocytes. *J Invest Dermatol* 1990; **94:** 310–16.

18 Morioka S, Lazarus GS, Jensen PJ. Involvement of urokinase-type plasminogen activator in acantholysis induced by pemphigus IgG. *J Invest Dermatol* 1987; **89:** 474–7.

19 Isseroff RR, Rifkin DB. Plasminogen is present in the basal layer of the epidermis. *J Invest Dermatol* 1983; **80:** 297–9.

20 Burge SM, Ryan TJ, Cederholm-Williams SA. Darier's disease: an immunohistochemical study using antibodies to proteases. *Br J Dermatol* 1989; **121:** 613–21.

21 Skerrow CJ. Plasmin induces pemphigus type acantholysis in normal skin. *Br J Dermatol* 1986; **113:** 765.

22 Hunziker Y, Vassalli J-D. Plasmin induces acantholysis in skin organ cultures. *Arch Dermatol Res* 1987; **279:** 341–6.

23 Baird J, Lazarus GS, Belin D *et al.* mRNA for tissue-type plasminogen activator is present in lesional epidermis from patients with psoriasis, pemphigus or bullous pemphigoid, but is not detected in normal epidermis. *J Invest Dermatol* 1990; **95:** 548–52.

24 Wiman B, Lijnen HR, Collen D. On the specific interaction between the lysine binding sites in plasmin and complementary sites in alpha-2-antiplasmin and in fibrinogen. *Biochim Biophys Acta* 1979; **579:** 142–59.

25 Deutsch DG, Mertz ET. Plasminogen: purification from human plasma by affinity chromatography. *Science* 1970; **170:** 1095–6.

26 Ishibashi Y, Ohtsuka F, Kukita A. Tissue culture of epidermal cells in some acantholytic dermatoses. *Curr Probl Dermatol* 1980; **10:** 91–111.

27 Ishibashi Y, Kukita A. Influence of cell dissociation on normal epidermal cells in Hailey–Hailey's disease and Darier's disease. *Curr Probl Dermatol* 1983; **11:** 59–68.

28 De Dobbeleer G, De Graef Ch, M'Poudi E *et al.* Reproduction of the characteristic morphologic changes of familial benign chronic pemphigus in cultures of lesional keratinocytes onto dead deepidermized dermis. *J Am Acad Dermatol* 1989; **21:** 961–5.

29 Regnier M, Ortonne J-P, Darmon M. Histological defects of chronic benign familial pemphigus expressed in tissue culture. *Arch Dermatol Res* 1990; **281:** 538–40.

30 Watt FM, Mattey DL, Garrod DR. Calcium induced reorganisation

of desmosomal components in cultured human keratinocytes. *J Cell Biol* 1984; **99**: 2211–15.

31 Mattey DL, Garrod DR Splitting and internalisation of the desmosomes of cultured kidney epithelial cells by reduction in calcium concentration. *J Cell Sci* 1986; **85**: 113–24.

32 Mattey DL, Garrod DR. Calcium-induced desmosome formation in cultured kidney epithelial cells. *J Cell Sci* 1986; **85**: 95–111.

33 Duden R, Franke WW. Organization of desmosomal plaque proteins in cells growing at low calcium concentrations. *J Cell Biol* 1988; **107**: 1049–63.

34 Penn EJ, Burdett IDJ, Hobson C, Magee AI. Structure and assembly of desmosomal junctions: biosynthesis and turnover of the major desmosome components of Madin–Darby canine kidney cells in low calcium medium. *J Cell Biol* 1987, **106**: 2327–34.

35 Boyer B, Tucker GC, Valles AM *et al.* Rearrangements of desmosomal and cytoskeletal proteins during the transition from epithelial to fibroblastoid organization in cultured rat bladder carcinoma cells *J Cell Biol* 1989; **109**: 1495–509

36 Felez J, Miles LA, Plescia J, Plow EF. Regulation of plasminogen receptor expression on human monocytes and monocytoid cell lines. *J Cell Biol* 1990, **111**: 1673–83.

37 Fibbi G, Magnelli L, Pucci M, Del Rosso M. Interaction of urokinase A chain with the receptor of human keratinocytes stimulates release of urokinase-like plasminogen activator *Expt Cell Res* 1990; **187**: 33–8.

38 Botteri FM, Ballmer-Hofer K, Rajput B, Nagamine Y. Disruption of cytoskeletal structures results in the induction of the urokinase-type plasminogen activator gene expression. *J Biol Chem* 1990; **22**: 13327–34.

British Journal of Dermatology (1991) **125**, 436–442.

Diurnal variations in lower leg subcutaneous blood flow rate in patients with chronic venous leg ulcers

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Accepted for publication 28 May 1991

Summary

The blood flow rate in subcutaneous adipose tissue was measured on the lower legs of 11 patients with chronic lower-leg venous insufficiency and ulceration and in eight age-matched control subjects for 12–20 h, under ambulatory conditions, using the ^{133}Xe wash-out technique with portable Cadmium telluride (CdTe(Cl)) detectors. In both groups, the change from an upright to a supine position at the beginning of the night period elicited an instantaneous increment in the blood flow rate of 30–40% with a decrease in the central and local postural sympathetic vasoconstrictor activity. After approximately 1 h of sleep, a considerable increase in blood flow rate was seen in both patient and control groups which persisted for nearly 100 min. In the patient group, the mean increase was 137% compared to a mean increase of 68% in the control group ($P<0{\cdot}01$). The blood flow then returned to the same level as at the beginning of the night and remained stable until the subjects woke in the morning. The differences between this nocturnal hyperaemic phase and the adjacent phases were highly significant in both groups ($P<0{\cdot}0001$ and $P<0{\cdot}005$, respectively). The blood flow levels measured on the second day were of the same magnitude as those registered on the first day. The mean values of the wash-out rate constants (k) of the two groups were of the same magnitude in all phases of subcutaneous blood flow level, except in the hyperaemic phase, where it was significantly higher in the patient group ($P<0{\cdot}01$).

The pattern of the nocturnal blood flow rate corresponded to that described previously in a younger healthy population, but with quantitative differences, which suggests that the abnormality occurs in the neural, humoral or metabolic factors involved in the nocturnal hyperaemic response.

Determination of local blood flow rates by means of the local radioisotope wash-out technique was described by Kety 40 years ago.[1,2] Applying the lipophilic tracer ^{133}Xe, a method was established for determination of absolute blood flow rates and relative changes in blood flow rates in cutaneous and subcutaneous tissue.[3–5] The method has been widely used in a number of clinical conditions using a stationary NaI(Tl) detector system for measurements over short periods of time under laboratory conditions. The innovation of a portable CdTe(Cl) detector system allowed prolonged measurements under out-patient conditions.[6–8]

In recent studies, we have performed measurements of subcutaneous blood flow rates over 12–16 h under ambulatory conditions on the medial and distal aspect of the right lower legs of normal human subjects. An atraumatic, epicutaneous labelling technique and the portable CdTe(Cl) detector system have been used.[9,10] Characteristic nocturnal fluctuations were observed. The change from an upright to a supine position on day 1 at the beginning of the night period elicited an increment in the blood flow rate of 30–40% in accordance with a decrease in central and local postural sympathetic vasoconstrictor activity. About 90 min later, there was an additional blood flow rate increment of considerable magnitude. The mean increase was 84%, but in several cases, an increment >200% was measured. The duration of this nocturnal hyperaemic phase was 100 min and a possible relationship with neurogenic or thermoregulatory adjustments and/or active cutaneous/subcutaneous vasodilation during sleep has been proposed.[9]

There is still disagreement as to the pathogenesis of lower-leg venous ulcers. Some authors have focused on morphological changes in the pericapillary bed due to sustained venous hypertension causing impaired oxygen delivery to the skin and subsequent cutaneous/subcutaneous changes.[11–13] Others have focused on

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neurogenic microcirculatory adjustments to changes in the venous intraluminal pressure.[14–16] In a recent study, we found that the activation/deactivation of the local veno-arteriolar axon reflex in lower leg subcutaneous tissue was preserved during the change from passive dependency to exercise on walking in patients with phlebographically verified chronic venous insufficiency.[17]

The present study was performed to determine whether the nocturnal regulation of subcutaneous blood flow was normal in patients with chronic venous insufficiency and chronic venous leg ulcers.

Methods

The study group comprised 11 patients with chronic venous ulceration of the lower leg (aged 66·0 ± 13·1 years, mean, SD). Five were female (aged 66·0 ± 14·7 years) and six were male (aged 66·0 ± 13·1 years). The patients had unilateral or bilateral ulceration of the legs and all the ulcers had been present for more than 2 months (16·7 ± 12·0 months, mean, SD). The systolic arm blood pressure was measured using a sphygmomanometer and the systolic ankle and toe blood pressures by the strain-gauge technique.[18] In all patients, the systolic ankle/arm index of the examined leg was $\geq 0{\cdot}90$ mmHg and the systolic toe blood pressure > 60 mmHg. All patients had phlebographic evidence of incompetent perforating veins in the examined leg.

Eight age-matched, healthy subjects served as controls (aged 63·1 ± 16·6 years). Two were female (aged 80·5 ± 10·6 years) and six were male (aged 57·3 ± 14·3 years). None of the patients or controls were receiving calcium antagonists, β-blocking agents or angiotensin-converting enzyme inhibitors. None had chronic heart failure, diabetes mellitus, and none had had hypertension or any symptoms or signs indicating peripheral atherosclerosis.

The study was approved by the local ethical committee and informed consent was obtained from the patients and controls.

Experimental procedure

In the patients with chronic venous leg ulcers, the blood flow measurements were performed on the medial aspect of the ulcer-bearing lower leg. The measurements were performed proximal to and in the immediate vicinity of the ulcer. All the selected patients had ulcers located on the medial aspect of the lower leg, and if bilateral ulceration was present, the right lower leg was chosen. In the healthy control subjects, the blood flow measurements were performed on the medial aspect of the right lower leg 10 cm proximal to the malleolar level.

One millilitre of a saline solution of the gas ^{133}Xe (5 mCi = 185 MBq) was injected into a gas-tight (Mylar membrane) chamber attached to the surface of the skin by means of double-sided adhesive tape. The exposure time was between 60 and 120 s during which diffusion of the tracer through the epidermis and convective transport by blood flow through the dermis to subcutis took place. The solution was drawn back into the syringe and the surplus of ^{133}Xe was dabbed off and blown away. The diameter of the labelled skin area was 4·0 cm. This atraumatic, epicutaneous labelling technique has been previously described.[3,5,8] Portable CdTe(Cl) semiconductor detectors were mounted over the central part of the labelled area. The detectors were fixed directly on to the skin surface by means of adhesive tape, which has been found to have no influence on the ^{133}Xe disappearance measurements.[6] A single layer of Mylar membrane was interposed between the detector and the skin surface to avoid accumulation of ^{133}Xe in the rubber cap of the detector during the measurement.[10] As the detectors weigh about 25 g each, and the fixation was made with care, pressure-induced influence on subcutaneous adipose tissue blood flow rate could be assumed to be of no significance.

The measurements were not commenced until at least 90 min after the labelling procedure in order to allow the lipophilic tracer to be deposited primarily in the subcutaneous adipose tissue.[4,10] The wash-out rate constants from the subcutaneous depot of ^{133}Xe were recorded continuously by the Memolog 500 or 600 system (B. Simonsen Medical A/S, Randers, Denmark) as previously described.[19] During measurement, counts were accumulated at 1-, 2- or 4-min intervals in a portable-data storage unit. The duration of each measurement was between 12 and 20 h, depending on the magnitude of the depot applied and on the blood flow rate. Typically, a measurement started at approximately 3:00 p.m. and ended at 10:00 a.m. the following day. During this period, which took place under ambulatory conditions, the subjects recorded their physical activities, time of meals and sleeping periods. The subjects performed their normal activities during daytime (standing, walking and sitting) and slept in a supine position at night. The subjects were not allowed to drink alcohol.

Analysis of data

The counts collected were computed and the logarithmically transformed clearance curves from relevant time periods were plotted. Linear regression analysis was performed, and only rate constants (k) from curve sections with a correlation coefficient (r) $>0{\cdot}80$ were taken into consideration. A few, minor curve sections were rejected on the basis of this criterion; however, in no cases were all data from an experiment omitted. Irregularities on the curves were typically due to either geometrical disturbances between the detector and the isotope depot or temporary detector circuit noise.

The curves were analysed for goodness of fit and linearity by linear regression analysis, and the distribution of the residuals were inspected on a plot. According to the former, the majority of the curves could be divided into five phases as follows: phase 1 = day 1, phase 2, 3 and 4 = night, phase 5 = day 2. Phase 2 was the initial supine period in bed after standing and was without the influence of any central or local postural vasoconstrictor activity. This phase was chosen as the reference level (Index = 1·00) and the blood flow levels during the other phases of measurement were calculated relative to phase 2.

During the night period, three distinct phases were detected, with highly significantly different isotope wash-out rates (phases 2, 3 and 4). The two break points between the three nocturnal phases were not associated with any known events. The determination of these two break points was not based solely on visual inspection. First the logarithmically transformed wash-out curve for the night period was inspected, then the residuals were generated and inspected on a plot.[9] The approximate location of the points on the wash-out curves where the wash-out rate constant changed (the break points) corresponded to the inflexions on the residual plot. The accurate location of the break points (time/count number on x-axis) was subsequently determined by computerized multiregression analysis by means of the least sum of squares.[9] The subcutaneous blood flow rate (SBF) can be calculated from:

$$\mathrm{SBF} = \lambda \times k \times 100\ \mathrm{ml} \times (\mathrm{min} \times 100\ \mathrm{g})^{-1}$$

Lambda ($\lambda = 10\ \mathrm{ml} \times \mathrm{g}^{-1}$ in normal subcutaneous tissue) denotes the tissue-to-blood partition coefficient of the tracer in the tissue investigated. Assuming λ to be constant throughout the measurement, relative changes in the wash-out rate constant (k) reflect relative changes in the SBF.

The levels of significance between the different blood flow rate phases within the two groups were determined by means of the Student's t-test for paired samples (SPSS). The independent-samples t-test (SPSS) was used for comparisons of phases between the two groups. A P value $<0{\cdot}05$ was considered significant.

Results

A logarithmically transformed ^{133}Xe wash-out curve from a 13-h day/overnight measurement on the lower leg of a patient with chronic venous insufficiency is shown in Figure 1. A similar curve recorded from the lower leg of an age-matched normal human subject is shown in Figure 2. The blood flow rate patterns of the two subjects were identical. It is evident that the night period could be divided into three separate curve segments with different slopes.

The mean indices and durations of the different phases in 11 patients with chronic venous ulcer and in eight age-matched controls are shown in Table 1. The blood-flow-rate pattern of the two groups was identical. Approximately 1 h after the subjects went to sleep, the blood flow rate suddenly increased by 137% in the patient group and by 68% in the control group. This difference between the patients and the controls was statistically significant ($P<0{\cdot}01$). In both groups, the duration of this hyperaemic phase was approximately 100 min, after which the blood flow rate returned to the prehyperaemic level. Also, in both groups, highly significant differences were found between the blood flow rate indices of phase 2 and 3 as well as between phase 3 and 4 ($P<0{\cdot}0001$ and $P<0{\cdot}005$, respectively). No significant

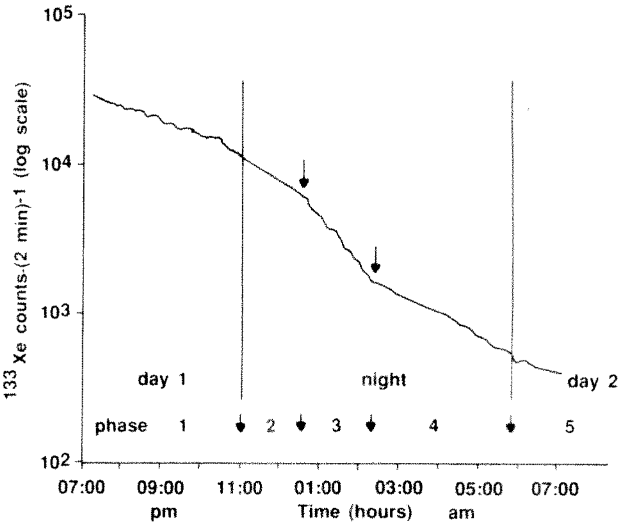

Figure 1. Logarithmically transformed ^{133}Xe clearance curve recorded over 13 h from a depot on the lower leg of a patient with chronic venous insufficiency. A characteristic three-phasic isotope wash-out rate pattern can be seen during the night period. Highly significant differences were found between the wash-out rates of phases 2 and 3 as well as between phases 3 and 4 ($P<0{\cdot}0001$). No significant difference was found between phases 1 and 5. The points of significant change in the wash-out rates are indicated by small arrows on the curve.

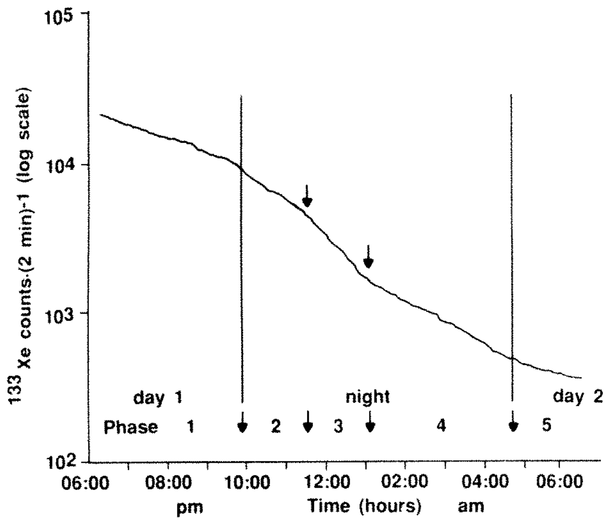

Figure 2. Logarithmically transformed ^{133}Xe clearance curve recorded over 12 h from a depot on the lower leg of a normal human subject. A characteristic three-phasic isotope wash-out rate pattern during the night period can be observed. Highly significant differences were found between the wash-out rates of phases 2 and 3 ($P<0{\cdot}005$) as well as between phases 3 and 4 ($P<0{\cdot}0001$). No significant difference was found between phases 1 and 5. The points of significant change in the wash-out rates are indicated by small arrows on the curve.

difference was found between the indices of phase 1 and phase 5 (day 1 and day 2) in either of the groups ($P=0{\cdot}41$ and $P=0{\cdot}47$, respectively). Thus, the blood flow levels on the first and on the second day were identical, and no further significant changes could be demonstrated in the blood flow levels. The mean values of the ^{133}Xe wash-out rate constants (k) in the different phases of lower leg subcutaneous blood flow rates in the patient and the control groups are shown in Table 2. The wash-out rate constants (k) of the two groups were of the same magnitude in all phases, except in the hyperaemic phase (phase 3), where it was significantly higher in the patient group ($P<0{\cdot}01$).

Discussion

The present study was carried out to investigate the regulation of the diurnal subcutaneous blood flow in the lower legs of patients with severe chronic venous insufficiency, i.e. chronic lower leg ulceration. Competing peripheral atherosclerosis was ruled out by the normal systolic ankle and toe pressures in the patient group. An identical diurnal subcutaneous blood flow

Table 1. Indices and durations of different phases of subcutaneous blood flow rate in the lower legs of 11 patients with chronic venous leg ulcers and eight normal human subjects

	Phase 1 (day 1)	Phase 2 (night)	Phase 3 (night)	Phase 4 (night)	Phase 5 (day 2)
Ulcer group					
Index	0·84±0·19	1·00	2·37±0·53**	1·10±0·56**	0·93±0·38
Duration (min)	122±83	70±57	108±60	233±113	118±96
Controls					
Index	0·72±0·14	1·00	1·68±0·40*	0·97±0·38**	0·66±0·05
Duration (min)	128±88	56±31	115±45	238±100	104±65

Values are mean ± SD. *$P<0{\cdot}005$, **$P<0{\cdot}0001$, compared to previous phase. Phase 1 = day 1 (walking, standing and sitting), phase 2 = start of night (supine rest in bed: reference level), phase 3 = hyperaemic phase (asleep in bed), phase 4 = rest of night (asleep in bed), phase 5 = day 2 (walking, standing and sitting).

Table 2. Mean values of the ^{133}Xe wash-out rate constant (k) in the different phases of subcutaneous blood flow rate in the lower legs of 11 patients with venous leg ulcers and in eight age-matched control subjects

	Phase 1 (day 1)	Phase 2 (night)	Phase 3 (night)	Phase 4 (night)	Phase 5 (day 2)
k (ulcer group) ($\times 10^{-3}$/min)	3·6±1·9	4·4±2·4	10·9±5·3	4·2±1·9	3·4±1·6
k (control group) ($\times 10^{-3}$/min)	3·1±1·4	3·8±1·9	6·2±2·5	3·9±1·2	2·2±1·2

Values are mean ± SD. The phases are explained in Table 1.

rate pattern was demonstrated in the lower legs of patients with chronic venous insufficiency and in an age-matched control group. Three significantly different blood flow levels were discovered during the night period. The change from an upright to a supine position on day 1 at the beginning of the night period elicited an instantaneous increase in the blood flow rate of approximately 30–40%. This is in accordance with the expected changes in blood flow rate due to deactivation of central and local sympathetic reflexes and humoral vasoconstrictor factors during orthostatic manoeuvres.[15,20,21] Furthermore, it is in accordance with our recent study of the lower leg subcutaneous blood flow during walking and passive dependency in chronic venous insufficiency.[17]

After approximately 1 h of sleep, a considerable increase in blood flow rate was seen in both groups. In some individuals there was an abrupt twofold elevation of the blood flow level which persisted for nearly 100 min. The blood flow subsequently returned to the same level as at the beginning of the night and remained stable until the subjects woke in the morning. The blood flow levels measured on the second day were of the same magnitude as those registered on the first day.

However, there were quantitative differences, as the ulcer patients had a significantly higher blood flow level during the nocturnal hyperaemic phase compared to the age-matched controls. The preceding and the succeeding blood flow levels were of the same magnitude in the two groups, estimated from the wash-out rate constants. Absolute blood flow rates should not be calculated for the ulcer patients without determination of the tissue-to-blood partition coefficient (λ) of the tracer in the particular tissue investigated. Lambda in the lipodermato-sclerotic tissue of the patients with chronic venous leg ulcers is expected to be lower than that known in healthy controls, but this difference alone could not account for the measured relative difference in the nocturnal hyperaemia response. This finding might indicate a state of capillary hypertension in the patients during the nocturnal hyperaemic phase, with subsequent induction of morphological and functional changes in the capillary bed of the patients with chronic lower-leg venous insufficiency. It might also be related to an abnormality in subcutaneous metabolism (oxygen extraction) or might be secondary to morphological and functional changes in the vasculature. The present findings of hyperaemia are, however, in contrast to the findings of Cherry *et al.*,[22] who in a recent study demonstrated reduced post-ischaemic (post-occlusive) hyperaemic responses and an increased time to peak hyperaemia by laser-Doppler fluxmetry in a corresponding patient group.

The eliciting mechanisms of these nocturnal fluctuations in subcutaneous blood flow levels are unknown. The sudden increase in blood flow rate points to a change in regulatory mechanisms within a very short time period. Four different eliciting mechanisms may be implicated: (i) local and/or central thermoregulation; (ii) local metabolic (myogenic) factor(s); (iii) local or central neurogenic control; or (iv) humoral factor(s). The possible significance of local thermal influences in the area under investigation were ruled out in a previous study, where measurements were performed over 6 h in the daytime with the subjects awake in the supine position. We were unable to demonstrate any hyperaemic phases under these conditions.[9] Thus the observed hyperaemic phase is not related to local thermal changes but seems to be related to other physiological events during or due to sleep.

In a previous study we found a highly significant positive correlation between the latency periods (phase 2) to the onset of the nocturnal hyperaemic phase (phase 3) during simultaneous measurements on identical locations on the right and left lower legs (J.H.Sindrup *et al.*, unpublished data). This may point to a central adjustment of cardiovascular regulatory mechanisms during sleep, although local metabolic factors cannot be excluded. If centrally elicited, the adjustment may be neurogenic or humoral and may possibly be related to central thermoregulation.[23] Vasomotor tone in skin and muscle tissue is known to be modulated by supraspinal and spinal sympathetic reflexes as well as a local sympathetic veno-arteriolar axon reflex.[15,20,24]

During the night, great fluctuations can be observed in many cardiovascular factors such as systemic blood pressure, heart rate, catecholamines, angiotensin II and thermoregulation,[25] and each of these components can potentially influence the blood flow level in subcutaneous tissue. The nocturnal increase in subcutaneous blood flow rate was of a high magnitude. Five minutes of total ischaemia was shown to be necessary to produce identical increments in the subcutaneous blood flow rate in the initial post-ischaemic hyperaemic phase, but 2·5 min after the ischaemia period, the blood flow rate had normalized.[26] In the present study, the hyperaemic period lasted approximately 100 min. Our data points to a resetting of the autoregulation curve towards a higher blood flow level during this period of the night. This is especially interesting, because systemic blood pressure is expected to decrease by 15–20% during this part of the sleeping period.[27,28] A similar resetting of the autoregulation curve has also been described in the brain during hypercapnia and hypoxia.[29-31]

The hyperaemic response in the age-matched controls was somewhat lower than that measured previously in a younger healthy population.[9] In that study, the inter-assay coefficients of variation were of the magnitude of 25–30%, which could partially explain this difference. However, the measured difference between normal subjects of different ages might suggest a weakening of the hyperaemic response with increasing age.

It appears that local as well as central postural regulating mechanisms are intact in patients with severe chronic venous insufficiency. The factors involved in the diurnal blood flow regulation show quantitative differences. This adds to the debate concerning the pathophysiological mechanisms for the tissue damage in lower leg venous hypertension.[12-15] A major role for the local veno-arteriolar axon reflex[14-16] in the pathogenesis of venous leg ulceration seems less probable from our studies.[17]

Acknowledgments

This work was supported by grants from the Danish Medical Research Council (grants no. 12-7357, 12-7888 and 12-7820), The Danish Heart Foundation, Danish Foundation for the Advancement of Medical Science, Novo's Foundation, Dagny and Harry West Jørgensen's Foundation, August Wedell Erichsen's Foundation, Jacob and Olga Madsen's Foundation, Valdemar and Thyra Foersom's Foundation. The authors wish to thank Mrs Birthe Christiansen and Mrs Lene Henriksen for practical and laboratory assistance and Coloplast a/s for providing that assistance and the Memolog system.

References

1 Kety SS. Measurement of regional circulation by the local clearance of radioactive sodium. *Am Heart J* 1949; 38: 321

2 Kety SS. Theory and application of the exchange of inert gas at the lungs and tissues *Pharmacol Rev* 1951, 3: 1.

3 Sejrsen P. Atraumatic local labelling of skin by inert gas. epicutaneous application of xenon 133. *J Appl Physiol* 1968; 4: 570.

4 Sejrsen P. Blood flow in cutaneous tissue in man, studied by washout of radioactive xenon. *Circulation Research* 1969; 15: 215

5 Sejrsen P. Measurements of cutaneous blood flow by freely diffusible radioactive isotopes, *Danish Med Bull* 1971; Suppl 18: 9–38.

6 Bojsen J, Kølendorf K, Staberg B Comparison of portable CdTe(Cl) detectors with stationary NaI(Tl) detectors for subcutaneous 133 Xenon disappearance measurements *Clin Physiol* 1983; 3: 325.

7 Bojsen J, Staberg B, Kølendorf K. Subcutaneous measurements of 133-xenon disappearance with portable CdTe(Cl) detectors: elimination of interference from combined convection and diffusion. *Clin Physiol* 1984; 4: 309.

8 Bojsen J. Investigations of portable cadmium telluride (CdTe(Cl)) detectors for clinical studies with radioactive indicators *PhD Thesis*, University of Copenhagen 1985; 6–56.

9 Sindrup JH, Kastrup J, Jørgensen B *et al.* Nocturnal variations in subcutaneous blood flow rate in the lower leg of normal human subjects. *Am J Physiol* 1991; 260: 480–5.

10 Sindrup JH, Kastrup J, Jørgensen B *et al.* Ambulatory measurement of nocturnal fluctuations in subcutaneous blood flow rate in the lower leg of man during 12 hour periods with the portable CdTe(Cl) detector. Methodological considerations. *Clin Physiol* 1991; 11: 353–65

11 Burnand KG, Whimster I, Clemenson G *et al.* The relationship between the number of capillaries in the skin of the venous ulcer-bearing area of the lower leg and the fall in foot vein pressure during exercise. *Br J Surg* 1981; 68: 297–300.

12 Burnand KG, Whimster I, Naidoo A, Browse NL. Pericapillary fibrin in the ulcer-bearing skin of the leg: the cause of lipodermatosclerosis and venous ulceration. *Br Med J* 1982; 285: 1071–2.

13 Browse NL, Burnand KG. The cause of venous ulceration. *Lancet* 1982; ii: 243–5.

14 Dodd HJ, Gaylarde PM, Sarkany I. Skin oxygen tension in venous insufficiency of the lower leg. *J R Soc Med* 1985, 78: 373–6.

15 Henriksen O. Local sympathetic reflex mechanism in regulation of blood flow in human subcutaneous adipose tissue *Acta Physiol Scand* 1977; Suppl. 450: 7–48.

16 Henriksen O, Sejrsen P. Effect of 'vein pump' activation upon venous pressure and blood flow in human subcutaneous tissue *Acta Physiol Scand* 1977; 100: 14–21.

17 Peters K, Sindrup JH, Petersen LJ *et al.* Lower leg subcutaneous blood flow rate during walking and passive dependency in chronic venous insufficiency. *Br J Dermatol* 1991; 124: 177–80.

18 Lassen NA, Tvedegaard E, Jeppesen EI *et al.* Distal blood pressure measurements in occlusive arterial disease, strain gauge compared to xenon-133. *Angiology* 1972; 23: 211.

19 Jelnes R, Bülow J Evaluation of a method for determination of the subcutaneous blood flow in the forefoot continuously over 24 hours. *Scand J Clin Lab Invest* 1984; 44: 85.

20 Mancia G, Mark AL. Cardiopulmonary vasoreflexes in humans. In: *Handbook of Physiology* (Shepherd JT, Abboud FM, eds). The cardiovascular system, Volume III. Peripheral circulation and organ blood flow, Part 2. Geigy S, Am Physiol Soc Baltimore: Williams and Wilkins, 1983; 795–813.

21 Rowell LB, Wyss CR, Brengelmann GL. Sustained human skin and muscle vasoconstriction with reduced baroreceptor activity. *J Appl Physiol* 1973; 5: 639

22 Cherry GW, Tanner R, Pepper J *et al.* Impairment in the microcirculation of patients with venous leg ulcers—a possible factor contributing to non-healing. Abstract *XVI European Conference on Microcirculation*, August 26–31, 1990. Zurich, Switzerland.

23 Rowell LB. Cardiovascular aspects of human thermoregulation. *Circulation Research* 1983; 4: 367.

24 Skagen K, Jensen K, Henriksen O, Knudsen L. Sympathetic reflex control of subcutaneous blood flow in tetraplegic man during postural changes. *Clin Sci* 1982; 62: 605.

25 Glotzbach SF, Craig Heller H. Thermoregulation In: *Principles and Practice of Sleep Medicine*. (M. H. Kryger, T. Roth, W C Dement, eds) Philadelphia: Saunders. 1989; 300–9.

26 Nielsen SL, Sejrsen P. Reactive hyperaemia in subcutaneous adipose tissue in man. *Acta Physiol Scand* 1972; 85: 71.

27 Mancia G, Ferrari A, Gregorini L *et al.* Blood pressure and heart rate variabilities in normotensive and hypertensive human beings. *Circulation Research* 1983; 53: 96.

28 Turjanmaa V, Kalli S, Majahalme S *et al.* Diurnal blood pressure profiles and variability in normotensive ambulant subjects. *Clin Physiol* 1987; 7: 389.

29 Busija DW, Heistad DD. Factors involved in the physiological regulation of the cerebral circulation. *Rev Physiol Pharmacol* 1984, 101: 161.

30 Kontos HA. Regulation of the cerebral circulation. *Ann Rev Physiol* 1981; 43: 497.

31 Raichle ME, Stone HL. Cerebral blood flow autoregulation and graded hypercapnia. *Europ Neurol* 1971/1972; 6: 1.

British Journal of Dermatology (1991) 125, 443–447.

Cross-reactivity between streptococcal M surface antigen and human skin

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Accepted for publication 28 May 1991

Summary Psoriasis can be triggered by haemolytic streptococcal infections. As M protein is a major pathogenic surface antigen in these streptococci, the cross-reactivity between streptococcal M protein surface antigens and human epidermis was investigated. The conserved component common to the few M proteins investigated consists of an alpha-helical 'coiled-coil' configuration, similar to sub-units of human keratin. The amino acid sequence of protein M6, one of the M proteins that has been fully sequenced, was compared with that of 4721 ubiquitous peptides, by computer-assisted analysis using a protein-sequence data bank. Of all human proteins in the data bank 50-kDa keratin type 1 showed the closest homology with protein M6. Further evaluation revealed that this homology mainly involved the heptapeptide repeat patterns, which form the alpha-helical 'coiled-coil' structure, in both M6 and 50-kDa keratin.

Cryostat sections of normal, involved and uninvolved psoriatic skin were studied for cross-reactivity with rabbit antisera raised against 10 different M proteins. All these antisera reacted with the stratum corneum of normal and psoriatic epidermis to a variable extent. Staining of keratinocyte cytoplasm was also observed, but this tended to be more prominent in lesional than in uninvolved and normal skin. Some of the M antisera also stained dendritic cells in the upper dermis as well as endothelium and smooth muscle. These cross-reactivities might be relevant to the pathogenesis of post-streptococcal psoriasis.

Beta-haemolytic streptococcal infection is a well known trigger of guttate psoriasis.[1] There have been few studies on the types of streptococci which are associated with this phenomenon, but as yet only streptococcal groups which express the M protein surface antigens (groups A, C and G) have been implicated.[2,3] Guttate psoriasis may precede chronic plaque psoriasis.[4] These clinical observations could be explained at least in part by immunological cross-reactivity between streptococcal M protein surface antigens and components in human skin. To investigate this further a comparison was made of amino-acid sequence homology between the M6 streptococcal surface antigen and 4721 other proteins with known sequences. Furthermore, antisera raised against 10 different M proteins were tested for reactivity against sections of human skin.

Methods

Homology analysis

The sequence of the M6 protein[5,6] was compared to a sequence data base of the protein identification resource (PIR).[7] The data base contains sequences of 4721 ubiquitous peptides, 1626 of which are mammalian. Proteins from plants, fungi, prokaryotes, animal and plant viruses and bacteriophages were included; one other M protein, M5, has been fully sequenced and this was included in the data base. Homology was expressed as the overall percentage of amino-acid sequences which were identical, and the homology was also analysed in terms of regional identities. The proteins were then ranked by a computer program ('Prosis', Hitachi American, CA, U.S.A.) according to their structural homology with protein M6. This program takes account of both the overall and the regional homology.

Assessment of immunological reactivity between M protein antisera and human skin

Skin samples. Nineteen punch biopsies (4-mm diameter) were obtained, five from normal skin, seven from uninvolved skin and seven from involved areas of psoriasis. Biopsies from the uninvolved psoriatic skin were taken at least 1 cm away from the lesions. All the

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samples were immediately frozen in tissue tek II OCT compound (Lamb, London, U.K.) and kept at −80°C.

M antisera. Pepsin extracts of M proteins were prepared by a pepsin digestion[8] and rabbit antisera against 10 different M proteins (M1, M2, M3, M6, M9, M12, M13, M22, M59 and M73) were obtained (Streptococcus Reference Laboratory, Central Public Health Laboratory, Colindale, London, U.K.). Dilutions of the whole sera (1/10–1/200) were used to stain the skin sections. Non-immune and anti-pneumococcal cell wall rabbit serum were used as controls. As a further control two skin sections of lupus erythematosus were stained with pep-M1 antisera.

Sections (4 μm) cut on a cryostat and air-dried for at least 30 min, were incubated for 30 min with the individual M-antisera and washed in PBS. Bound rabbit IgG was then detected using a standard avidin–biotin immunoperoxidase labelling technique according to the manufacturer's instructions (Dakopatt, Denmark). Further preparations were made using an immunofluorescent technique with an FITC-labelled swine anti-rabbit IgG antibody to detect binding of the M antisera to tissue sections. Reaction of the M antisera with dendritic cells was verified by a double-staining technique, using a mouse monoclonal anti-vimentin antibody and rhodamin conjugated anti-mouse IgG antibody (Dako) to identify the vimentin-positive cells.

Sensitivity and specificity of M antisera

For each antisera, the presence of antibodies against the particular pepsin M extract was demonstrated using a double immunodiffusion gel. Type-specific antibodies were demonstrated by opsonization of the particular M strain of streptococci in the presence of antisera.[9]

Results

The 20 proteins showing the closest homology with protein M6 are listed in Table 1. These included two human proteins and of these 50-kDa human type 1 keratin[10,11] ranked higher. One seven amino-acid (aa) sequence on M6 (Gly-Leu-Arg-Arg-Asp-Leu-Asp), corresponding to an epitope that has been identified by monoclonal antibody on two-thirds of other M proteins tested,[12] had only one aa site that differed from the corresponding 50-kDa keratin sequence (Gly-Leu-Arg-Arg-Val-Leu-Asp) (Table 2). Analysis of the secondary structure of both proteins at this and most other sites predicted that they would form an alpha-helical 'coiled-coil' structure (Fig. 1).

Table 1. Sequence homology between streptococcal M6 antigen and ubiquitous proteins ranked on the basis of both regional and overall homology

Rank	Protein	Species	Length aa overlaps compared	Percentage overall identity
1	M5	*Strep. pyogenes*	154	47·4
2	Myosin heavy chain	*C. elegans*	370	20·5
3	Myosin heavy chain I	*C. elegans*	145	24·8
4	Keratin, 54-kDa type I cytoskeletal	Bovine	161	19·3
5	Tropomyosin beta chain, platelet	Horse	201	16·9
6	Neurofilament triplet L protein	Pig	163	18·4
7	Keratin 50-kDa type I cytoskeletal	Human	223	18·4
8	Apolipoprotein A-IV precursor	Human	243	24·3
9	Keratin, 59-kDa type I cytoskeletal	Mouse	133	16·5
10	Myosin heavy chain 2 skeletal muscle	Rabbit	241	17·4
11	Myosin heavy chain I cardiac muscle	Rat	105	22·9
12	Myosin heavy chain 2 cardiac muscle	Rat	90	26·7
13	Myosin heavy chain fast skeletal muscle, embryonic	Chick	118	21·2
14	Transforming protein	Avian	95	21·1
15	Transforming protein MYC	Avian	95	23·2
16	Myosin beta heavy chain, cardiac muscle	Rabbit	271	20·3
17	Myosin alpha chain cardiac muscle	Rabbit	303	17·8
18	Histone HIB	African clawed frog	199	25·1
19	Apolipoprotein A-IV precursor	Rat	243	19·8
20	Tropomyosin alpha chain	Rabbit	221	18·6

Table 2. Amino-acid homology sequence profile between streptococcal protein M6 (M6) and 50-kDa type 1 keratin (KER)

```
                                                  ....KETIGT    M6
                                                      : :. .
                                                  ....KVTMQN    KER
LKKTLDETVKDKIAKEQESKETIGTLKKTLDETVKDKIAKEQESKETIGTLK-KILDETV    M6
... :.. ...  : :... .   ....  .    ..: .     .::..:. :::..::
LNDRLASYLDKVRALEEANADLEVKIRDWYQRQRPAEIKDYSPYFKTIEDLRNKILTATV    KER

KD--KIAREQKSKQDIGALKQELAKKDEGNKVSEASRKGLRRDLDASREAKKQVEKDLAN    M6
..   . . .... . .... . ... . ..  ::. .:::: ::.   :. ..:.....
DNANVLLQIDNARLAADDFRTKYETELNLRMSVEADINGLRRVLDELTLARADLEMQIES    KER

LTAELDKVKEEKQISDASRQGLRRDLDASREAKKQVEKALEEANSKLAALEKLNLELEES    M6
:..::. .   :.  ......:: ..... ....... ... ..   .  .. .:  :..
LKEELAYL---KKNHEEEMNALRGQVGGDVNVEMDAAPGVDLSRILNEMRDQYEKMABEN    KER

KKLTEKEKAELQAKLEAEAKALKEQLAKQAEELAKLR....                       M6
.: .:.   .  ..:. :. . .: ...  .:...::
RKDAEEWFFTKTEELNREVATNSELVQSGKSEISELR....                       KER
```

:, identical amino acid; · amino acids with similar hydrophobicity; boxed area –, known 'common' epitope site of M6 protein.

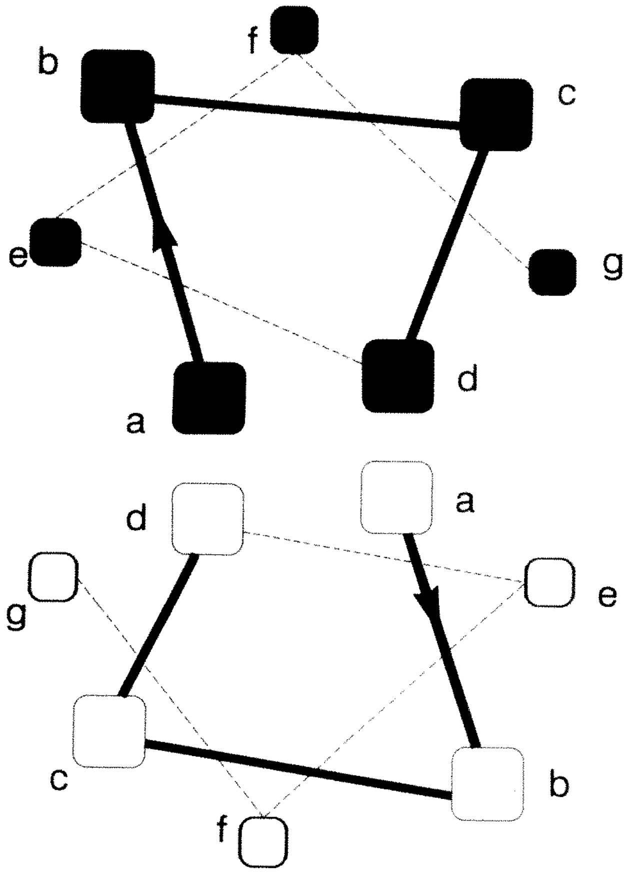

Figure 1. 'Coiled-coil' structure: letters 'a' to 'g' indicate sites of seven amino-acid periodicity.

Reactivity of M antiserum with human epidermis

The M antiserum preparations all reacted with components in human epidermis but individual antisera showed some variability in the localization and extent of staining. Some stained all layers of the epidermis and others seemed to react preferentially with the granular layer, basal cells or the basement membrane. Lesional psoriatic epidermis showed stronger and more extensive reaction than the normal epidermis (Fig. 2) and this applied to all the antisera tested. With non-immune and anti-pneumococcal rabbit serum, there was no reaction, apart from scattered staining of the stratum corneum. Whereas M1 antisera stained only the basal cell layer in lupus erythematosus there was staining throughout the epidermis in the psoriatic skin. Immunofluorescence studies demonstrated staining in the dermis of smooth muscle and vascular endothelium. Dermal dendritic cells, identified by double labelling with vimentin antibody, also reacted with at least some of the M antisera (M1 and M6). These cells were preferentially localized near the dermal–epidermal junction.

Discussion

This study reports homology between the streptococcal M6 protein and human keratin. Furthermore M proteins

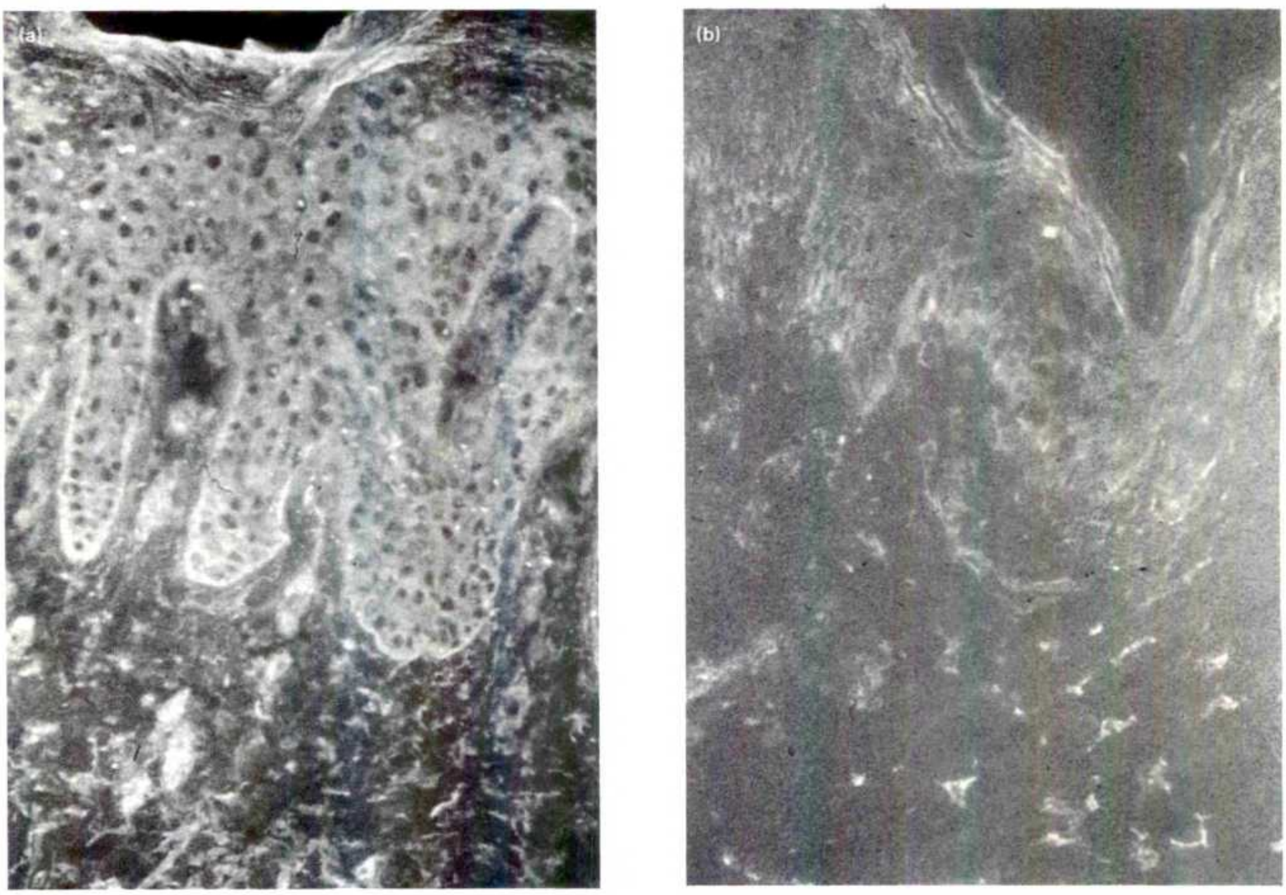

Figure 2. Immunofluorescent labelling of (a) psoriatic and (b) normal skin after incubation with 1/100 M6 antisera.

and components of human epidermis are immunologically cross-reactive when tested with polyclonal rabbit antibodies against M protein. The M protein is a surface antigen forming hair-like filaments that cover the surface of certain groups of haemolytic streptococci.[13] It is an important factor for virulence and is thought to interfere with phagocytosis. Antibodies to the M protein can prevent clinical infection.[14]

Because epidermal staining was noted with all the different M antisera preparations, it would appear that it is mainly the non-variable part of the M protein which cross-reacts with the epidermal components. This part of the M protein contains the alpha-helical 'coiled-coil' structure with the seven amino-acid residue periodicity, which is remarkably similar in configuration to subunits of human keratin. Based on a model by McLachlan and Stewart[15] (Fig. 1), the seven positions within each period are called a, b, c, d, e, f and g. The 'a' and 'd' ('core') positions are normally non-polar. The remaindder of the helix is generally polar and position e tends to be basic and g acidic. The alpha-helical 'coiled-coil' structure is particularly well suited to stable, elongated, projections that can be flexible.

The limited data available indicates that the ability of streptococci to trigger psoriasis is not restricted to certain M serotypes, suggesting that if the M protein is involved in this disease it is likely to be the conserved part of the molecule which is implicated, the common 'coiled-coil'. The enhanced cross-reactivity between the M protein and keratinocytes in lesional skin is possibly a secondary phenomenon to the abnormal keratin production seen in psoriasis.[16] It is of interest that the 50-kDa keratin, which has the closest human homology with M protein, is found in increased quantities and preferentially retained in psoriasis.[16] Elevated levels of antibodies to 50-kDa keratin have been demonstrated in psoriasis and which correlate with disease activity.[17] It has recently been reported that M protein is a superantigen;[18] the relevance of this with regard to post-streptococcal sequelae has yet to be determined, but cross-reactivity and superantigenic involvement are not necessarily mutually exclusive in the pathogenesis of the disease.

Triggering of psoriasis by streptococcal infection is a well-recognized clinical phenomenon. Antibodies cross-reacting between streptococcal and epidermal components have already been demonstrated.[19] We have postulated that psoriasis is a disease of abnormal keratinocyte proliferation which is mediated by T cells,[20] and the dramatic improvement of psoriasis following treatment with low-dose cyclosporin supports this hypothesis. Cross-reactivity at the T-cell level between streptococcal and human epidermal components has, to our knowledge, not been reported. However as T cells predominantly recognize primary aa sequences[21] the close regional aa sequence homology between M protein and keratin suggests that cross-reactivity may also operate at the T-cell level. Molecular mimicry between streptococcal and epidermal components may therefore exist to such a degree as to allow T-cell clones directed against streptococcal components to initiate the psoriatic process in genetically predisposed individuals.

Acknowledgments

We thank Dr G.Colman and Dr D.Morrison from the Streptococcus Reference Laboratory, Public Health Laboratory Service, London, for advice and provision of antisera.

References

1 Whyte JJ, Baughman RD. Acute guttate psoriasis and streptococcal infection. *Arch Dermatol* 1964; **89**: 350–6.

2 Norrlind R. Psoriasis following infections with haemolytic streptococci. *Acta Derm Venereol (Stockh)* 1950; **30**: 64–72.

3 Henderson CA, Highet AS. Acute psoriasis associated with Lancefield group C and G cutaneous streptococcal infections. *Br J Dermatol* 1988; **118**: 559–62.

4 Williams RC, McKenzie AW, Roger JH, Joysey VC. HL-A antigens in patients with guttate psoriasis. *Br J Dermatol* 1976; **95**: 163–7.

5 Scott JR, Pulliam WM, Holligshead SK, Fischetti VA. Relationship of M protein genes in group A streptococci. *Proc Natl Acad Sci* 1985: **82**: 1822–6.

6 Holingshead SK, Fischetti AV, Scott JR. Complete nucleotide sequence of type 6 M protein of the group A streptococcus: repetitive structure and membrane anchor. *J Biol Chem* 1986; **261**: 1677–86.

7 Barker WC, Hunt DG, George LS *et al.* Protein sequence database of the Protein Identification Resource (P.I.R.). National Biomedical Research Foundation, Georgetown University Medical Centre, Washington DC 20007, 1987.

8 Beachey EH, Campbell GL, Ofek I. Peptic digestion of streptococcal M protein. II. Extraction of M antigen from group A streptococci with pepsin. *Infect Immun* 1974; **9**: 891–6.

9 Rothbard S. Bacteriostatic effect of human sera on group A streptococci. *J Exp Med* 1945; **82**: 93–9.

10 Marchuk D, McCrochon S, Fuchs E. Remarkable conservation of structure among intermediate filament genes. *Cell* 1983; **34**: 491–8.

11 Hanukoglu I, Fuchs E. The cDNA sequence of a human epithelial keratin: Divergence of sequence but conservation of structure among intermediate filament proteins. *Cell* 1982; **31**: 243–52.

12 Jones KF, Khan SA, Erickson BW *et al.* Immunochemical localisation and amino acid sequences of cross-reactive epitopes within the group A streptococcal M6 protein. *J Exp Med* 1986; **164**: 1226–38.

13 Swanson J, Hsu KC, Gotschlich EC. Electron microscopic studies on streptococci. *J Exp Med* 1969; **130**: 1063–7.

14 Wannamaker LW, Denny FW, Perry WD *et al.* Studies on immunity to streptococcal infections in man. *Am J Dis Child* 1953; **86**: 347–8.

15 McLachlan AD, Stewart M. Tropomyosin: coiled-coil interactions: Evidence for an unstaggered structure. *J Mol Biol* 1975; **98**: 293–304.

16 Hunter I, Skerrow D. Relationship of the final alpha-keratin compositions in dyskeratosis to those along the normal keratinisation pathway: aetiological and diagnostic significance. *Br J Dermatol* 1989; **120**: 363–70.

17 Aoki S, Yaoti H, Kitajama Y. An elevated level of autoantibodies against 48- to 50-kD keratins in the serum of patients with psoriasis. *J Invest Dermatol* 1989; **92**: 179–83.

18 Tomai M, Kotb M, Majumdar G, Beachey EH. Superantigenicity of streptococcal M protein. *J Exp Med* 1990; **172**: 359–62.

19 Swerlick RA, Cunningham MW, Hall NK. Monoclonal antibodies cross-reactive with Group A streptococci and normal and psoriatic skin. *J Invest Dermatol* 1986; **87**: 367–71.

20 Valdimarsson H, Baker BS, Jonsdottir I, Fry L. Psoriasis: a disease of abnormal keratinocyte proliferation induced by T lymphocytes. *Immunology Today* 1986; **7**: 256–9.

21 Livingstone AM, Fathman CG. The structure of T cell epitopes. *Ann Rev Immunol* 1987; **5**: 477–501.

British Journal of Dermatology (1991) **125**, 448–451.

The prevalence of antinuclear antibodies in patients with apparent polymorphic light eruption

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Accepted for publication 17 June 1991

Summary

Polymorphic light eruption (PLE) is a very common photosensitive disorder, the most important differential diagnosis of which is lupus erythematosus (LE). One-hundred and forty-two patients with PLE were screened for circulating antinuclear (ANA), Ro and La antibodies over a 2-year period. Results were negative in 66 patients. Sixty-two patients had low-titre ANA of various patterns, ranging from trace to 1/80 without evidence of LE although one later developed subacute cutaneous LE. Fourteen had more significant findings, six with ANA ranging from 1/160 to 1/1280 but no anti-Ro antibodies, four with ANA ranging from 1/160 to 1/1280 and also with anti-Ro antibodies and four patients with anti-Ro antibodies but low-titre ANA, one of whom later developed discoid LE. Three of these 14 patients fulfilled the American Rheumatism Association criteria for the diagnosis of systemic LE, but it was not certain in any of the patients whether the PLE-like rash represented cutaneous LE or coincidental PLE. However the overall 10% incidence of definite or possible LE in patients with suspected PLE suggests that all PLE patients should be screened for LE.

Polymorphic light eruption (PLE) is a common photodermatosis affecting 10–20% of the population[1] and may be a delayed-type hypersensitivity response to an ultraviolet (UV) radiation-induced cutaneous neoantigen. There is a dermal cellular inflammatory infiltrate and expression of intercellular adhesion molecule-1 (ICAM-1) on keratinocytes which is consistent with that seen in delayed type hypersensitivity.[2,3] The diagnosis of PLE is based primarily on the typical clinical history, often in the absence of a visible rash, because the skin lesions are transient and many patients are not affected at the time of clinical presentation. Differential diagnoses suggested by the history alone include other photodermatoses and the light-exacerbated dermatoses and in particular lupus erythematosus (LE). It has therefore been our practice to exclude LE by routinely checking for antinuclear, anti-Ro and anti-La antibodies in all patients with apparent PLE presenting to the photobiology clinic. To determine the value of such screening we have now undertaken a retrospective study to assess the proportion of patients with an original presumptive diagnosis of PLE, based on clinical history alone, who have eventually proved to have laboratory evidence of LE.

Methods

Pathology records were studied over the 3-year period from January 1987 to December 1989. All request forms submitted during this time for assessment of antinuclear antibodies in patients with presumptive PLE who were seen in the photobiology clinics at St John's Hospital for Diseases of the Skin, London, were examined, the results noted, and the clinical notes retrieved to verify the original clinical diagnosis. With some patients, follow-up data of up to 2 years was available from the case notes. Because it was routine practice to screen all presumptive PLE patients for the possibility of LE, virtually all such patients during this time period were considered to have been included in the study.

Antinuclear antibodies (ANA) were identified by indirect immunofluorescence on rat-liver sections. Anti-Ro (SSA) and La (SSB) antibodies were identified by counter-immunoelectrophoresis. Although not strictly quantifiable, the distance of the precipitate from the serum well (proportional to the amount of antibody present) was graded as trace, +, ++, or +++.

Results

One-hundred and forty-two patients with an original clinical diagnosis of PLE were identified. Results were

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Table 1. Summary of clinical details, LE autoantibody status, and histological findings of patients with Ro antibodies

Patient	Age	Sex	Autoantibody	Histology	Clinical status
S M.	29	F	ANA 1/20 Ro + + +	No rash	Three episodes typical PLE referred to St John's for PUVA
*V.H.	40	F	ANA 1/20 Ro + + +	No rash	Arthritis, malar rash, 8 years Hx PLE, FH PLE
T.A.	27	F	ANA Trace Ro + +	Not done	5 years Hx and FH PLE, typical PLE rash present
J.G.	30	F	ANA 1/10 Ro trace	Typical of LE+IF	PLE 11 years, DLE on nose for 1 year
M.G.	50	F	ANA 1/160 Ro + +	No rash	8/12 Hx typical PLE
C B	16	M	ANA 1/640 Ro + + La + + +	Vasculitis	1 year Hx PLE, PLE-like rash present
*P.K.	47	F	ANA 1/1280 Ro + + La + + +	No rash	2 year Hx and FH PLE livedo, arthralgia, wcc 3
C.B.	42	F	ANA 1/160 Ro trace	No rash	2 year Hx typical PLE

Hx, clinical history; FH, family history; IF, direct immunofluorescence, *, SLE.

negative in 66 patients (mean age 35 years, range 8–63 years). Of the remaining 76, 62 had ANA of various patterns, ranging from trace amounts to titres of 1/80 (mean age 35 years, range 11–65 years). The other 14 had more significant findings: six with ANA ranging from 1/160 to 1/1280 but no anti-Ro antibodies; four with ANA ranging in titre from 1/160 to 1280 and also with anti-Ro antibodies, three of them strongly positive and two also with anti-La antibodies; and four with anti-Ro antibodies but with low-titre ANAs ranging from trace amounts to titres of 1/20. The clinical details of those with positive ANA and anti-Ro antibodies are summarized in Table 1 and those with significantly positive ANA and negative anti-Ro in Table 2.

Skin biopsies were carried out on five of the 14 patients with significant findings, and in one patient with ANA 1/10 and a persistent rash suggestive of LE. The remainder had not undergone skin biopsy because the rash was not present at the time of assessment (seven patients) or because the rash was considered so typical of PLE that biopsy was not thought necessary (two patients). Direct immunofluorescence was carried out in four of the six patients biopsied. Definite histological features of LE were present in four patients (Tables 1 and 2): one with ANA 1/10 and trace positive anti-Ro who developed discoid LE on the nose 10 years after developing a rash typical of PLE; another with ANA 1/640, strongly positive ribonucleoprotein (RNP) and negative anti-Ro antibodies who had skin histology typical of LE; a third with ANA 1/320 and negative anti-Ro who fulfilled the American Rheumatism Association criteria for SLE and whose skin biopsy demonstrated the typical histology of LE; and a fourth with ANA 1/10 but negative anti-Ro antibodies who was subsequently thought to have subacute cutaneous LE both clinically and histologically. A further patient, a 16-year-old boy, was thought clinically at first presentation to have persistent lesions of PLE; however, with an ANA of 1/640 and a strongly positive anti-Ro antibody titre together with histological evidence of leucocytoclastic vasculitis, the diagnosis of cutaneous LE was considered likely and he has since been well controlled with mepacrine (T. Cutler, personal communication).

Three of the 14 patients had evidence of systemic

Table 2. Summary of clinical, immunological and histological investigations in patients with negative Ro antibodies but positive ANA

Patient	Age	Sex	Autoantibody	Histology	Clinical status
E.G.	41	F	ANA 1/1280	No rash	8 years Hx typical PLE, referred for PUVA
H.W	61	F	ANA 1/320	No rash	Typical PLE, tolerance by autumn
E.Y.	45	F	ANA 1/160	Not done	4 years Hx typical PLE
R.S.	64	M	ANA 1/640 RNP + + +	Typical LE	8 years Hx PLE
F.N.	65	F	ANA 1/320 Thglb 1/100	Compatible with PLE – IF	Sun rash since age 12, hypothyroidism
*V.N.	45	F	ANA 1/320	Typical LE – IF	PLE 4 years, rash, alopecia, arthritis, malaise
S.B	29	F	ANA 1/10	SCLE + IF	PLE for 4 years, developed SCLE-like rash

Thglb, thyroglobulin; SCLE, subacute cutaneous LE; Hx, clinical history; IF, direct immunofluorescence; *, SLE.

involvement. One patient had a malar rash and arthritis, a second had proteinuria, arthralgia, livedo reticularis and a white cell count of 3×10^9/l (Table 1), and a third had malar rash, alopecia, arthritis, malaise and positive skin histology (Table 2). These patients all fulfilled the 1982 revised American Rheumatism Association criteria for systemic LE[4] because of their systemic involvement, photosensitivity and positive ANA, although all three had mild disease, anti-DNA antibodies within the normal range and no renal impairment.

Discussion

Lupus erythematosus affecting the skin is classified into acute LE, subacute cutaneous LE, chronic discoid LE and lupus profundus.[5] Although photosensitivity is a well-recognized feature of all types of LE, it is most frequently associated with subacute cutaneous LE in which the majority of patients have circulating anti-Ro antibodies.[6] The rash, classically linked with subacute cutaneous LE, is a papulosquamous polycyclic or psoriasiform eruption, which often persists for weeks in the absence of treatment.[6] Other forms of lupus may also be photosensitive,[7–10] for example neonatal LE, a consequence of passive maternal transmission of anti-Ro antibodies and chronic discoid LE,[9] in which UV-irradiation of the skin produces papulosquamous lesions in 50% of photosensitive patients.[9] Photosensitive lupus is unlikely to be confused with PLE in patients with established LE, but it is now clear that some cases of LE presenting with only a transient photosensitive rash cannot always be definitely distinguished on clinical grounds alone from PLE. In most PLE patients the morphology of the eruption is not seen at clinical consultation and because the time courses and morphology of both the LE and PLE rashes are often similar, the two conditions may be confused.

Thus of 142 patients presenting with a transient photosensitive rash indistinguishable from PLE, we have found that 15 had significant abnormalities confirming or strongly suggesting the diagnosis of LE. Three had SLE, one later developed DLE and one SCLE, one had leucocytoclastic vasculitis, photosensitivity and strongly positive ANA and anti-Ro antibodies making the likelihood of LE great and nine others had significantly abnormal ANA or Ro antibodies. Although in each case there is a strong possibility that the PLE-like rash represented lupus photosensitivity, it is also possible that in some or all of these patients the occurrence of two unrelated phenomena, namely LE and PLE, was coincidental, or that the high-titre ANA or Ro antibodies were occasionally spurious findings. Against the latter suggestion however is the fact that such high antibody titres are rarely seen in the general population. They are also virtually never seen in chronic actinic dermatitis, another photosensitive condition not resembling LE, in which it has been our practice to screen routinely for antinuclear and anticytoplasmic autoantibodies.

The role of antinuclear and anticytoplasmic autoantibodies in the pathogenesis of the photosensitivity of LE is unresolved. Anti-Ro antibodies are related temporally to the appearance and disappearance of the photosensitive rash of neonatal LE.[7,11] UVB-irradiation of cultured normal keratinocytes[12,13] and human skin *in vivo*[14] leads to expression of Ro antigen on the cell surface, probably leading to cytotoxic effects.[12] Anti-Ro antibodies are known to be associated with subacute cutaneous LE,[6] neonatal LE,[11] C_2 and C_4 deficiency[15,16] and ANA-negative LE, all characteristically photosensitive disorders.

Efforts to associate the presence of circulating ANA with epidermal damage have not been rewarding.[17,18] This may be because UV irradiation is also necessary to alter skin antigenically, immunoreactants being frequently detected in chronically light-exposed areas in SLE.[19] However, UV irradiation of genetically susceptible mice and humans induces or exacerbates LE.[20]

In order to clarify whether our group of apparent PLE patients with high-titre ANA or Ro antibodies have LE, further cutaneous UV provocative studies are underway to asssess whether differentiation from ANA- and Ro-negative PLE is possible. Long-term follow-up of the patients is also being undertaken to define more fully the disease course and prognosis.

Acknowledgment

We are most grateful to Ms Jill Gilroy, The Autoimmune Serology Laboratory, The Academic Department of Immunology, Royal Free Hospital, London, who did the autoantibody studies and provided details of methodology.

References

1 Ros A, Wennersten G. Current aspects of polymorphous light eruptions in Sweden. *Photodermatol* 1987; **3**: 298–302.
2 Moncada F, Gonzales-Amara R, Baranda ML *et al.* Immunopathology of polymorphous light eruption. T lymphocytes in blood and skin. *J Am Acad Dermatol* 1984; **10**: 970–3.
3 Norris PG, Morris J, Chu AC, Hawk JLM. Polymorphic light eruption: an immunopathological study of evolving lesions. *Br J Dermatol* 1987; **118**: 287.
4 Tan EM, Cohen AS, Fries J *et al.* The 1982 revised criteria for the

classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**: 1271–7.
5 Wechsler HL. Cutaneous disease in systemic lupus erythematosus. *Clin Dermatol* 1985; **3**: 79–87.
6 Sontheimer RD, Thomas JR, Gilliam JN. Subacute cutaneous lupus erythematosus: a cutaneous marker for a distinct lupus erythematosus subset. *Arch Dermatol* 1979; **115**: 1409–15.
7 Lee LA, Lillis PJ, Fritz KA. Neonatal lupus syndrome in successive pregnancies. *J Am Acad Dermatol* 1983; **9**: 401–6.
8 Cripps DJ, Rankin J. Action spectra of lupus erythematosus and experimental immunofluorescence. *Arch Dermatol* 1973; **107**: 563–8.
9 Freeman RG, Knox JM, Owens DW. Cutaneous lesions of lupus erythematosus induced by monochromatic light. *Arch Dermatol* 1969; **100**: 677–82.
10 Epstein JH, Tuffanelli DL, Dubois EL. Light sensitivity and lupus erythematosus. *Arch Dermatol* 1965; **91**: 483–5.
11 Mond CB, Peterson MGE, Rothfield NF. Correlation of anti-Ro antibody with photosensitivity rash in systemic lupus erythematosus patients. *Arthritis Rheum* 1989; **32**: 202–4.
12 LeFeber WP, Norris DA, Ryan SS *et al.* Ultraviolet light induces binding of autoantibodies to selected nuclear antigens on cultured human keratinocytes. *J Clin Invest* 1984; **74**: 1545–51.
13 Norris DA, Ryan SR, Fritz KA *et al.* The role of RNP, Sm and SSA/Ro specific antisera from patients with lupus erythematosus in inducing antibody dependent cellular cytotoxicity (ADCC) of targets coated with non-histone nuclear antigens. *Clin Immunol Immunopathol* 1984; **31**: 311–20.
14 Furukawa F, Kashihara-Sawami M, Lyons MB, Norris DA. Binding of antibodies to the extractable nuclear antigens SS-A/Ro and SS-B/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): Implications for the pathogenesis of photosensitive cutaneous lupus. *J Invest Dermatol* 1990; **94**: 77–85.
15 Vandersteen PR, Provost TT, Jordan RE, McDuffie FC. C_2 deficient systemic lupus erythematosus: its association with anti-Ro(SSA) antibodies. *Arch Dermatol* 1982; **118**: 584–87.
16 Tappeiner G, Hinter H, Scholz S *et al.* Systemic lupus erythematosus in hereditary deficiency of the fourth component of complement. *J Am Acad Dermatol* 1982, **7**: 66–79.
17 Ward JR, Cloud RS, Turner LM Jr. Non cytotoxicity of nuclear antibodies from lupus erythematosus sera in tissue culture. *Ann Rheum Dis* 1961; **23**: 381–92.
18 Marmot AM. The transfusion of active lupus erythematosus plasma into non-lupus recipients with a note on the LE-like cell. *Ann NY Acad Sci USA* 1965; **124**: 838–45.
19 Kerker BJ, Morison WL. The photoaggravated dermatoses. *Sem Dermatol* 1990; **9**: 70–7.
20 Ansel JC, Mountz J, Steinberg AD *et al.* Effects of UV radiation on autoimmune strains of mice: increased mortality and accelerated autoimmunity in BXSB male mice. *J Invest Dermatol* 1985; **85**: 181–6.

British Journal of Dermatology (1991) **125**, 452–455.

Taking the 'sting' out of local anaesthetics

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Accepted for publication 28 May 1991

Summary In order to investigate factors influencing the discomfort caused by the injection of different lignocaine preparations, a randomized, double-blind comparison of paired injections was performed in 32 patients. In all subjects 2% plain lignocaine was found to be more painful than 0·5% plain lignocaine. Lignocaine with adrenaline 1:200,000 was found to be significantly more painful than plain lignocaine. The presence of sodium metabisulphite (the antioxidant in commercial adrenalized lignocaine) significantly increased the discomfort. Neutralization of acidic 0·5% lignocaine (pH 4·7) reduced the discomfort caused in 44% of patients, but this was not statistically significant.

Many procedures such as the suturing of lacerations, minor surgery, percutaneous biopsy, and the insertion of venous cannuale are performed with local anaesthesia. Lignocaine is the most widely used preparation and provides a rapid onset and a prolonged duration of action.[1] However, the injection of lignocaine is associated with a burning sensation,[2–11] and this is often the most uncomfortable part of the procedure.

A number of factors are known to influence the discomfort of injections of local anaesthetics. The pain of needle puncture may be reduced by the use of fine-gauge needles, but some anatomical sites such as the sole of the foot are intrinsically more painful than others. Stretching or vibrating the skin at the injection site makes injection less painful.[2] Injections within the dermis are more painful than subcutaneous injections and rapid injections more painful than slow ones. Pre-warming the local anaesthetic to body temperature does not appear to make a difference.[3] These factors may all be taken into account by the doctor giving the injection.

Lignocaine is a weak base that is prepared in aqueous solution as the soluble hydrochloride salt.[1] Commercial preparations of lignocaine have an acidic pH of between 3·82 and 6·4.[12] Aqueous solutions of lignocaine are rendered isotonic by the addition of sodium chloride and multidose vials also contain the antimicrobial methylparabenzoate. Adrenaline is added to lignocaine to provide local vasoconstriction. This has the benefit of reducing bleeding, prolonging anaesthesia, and delaying systemic absorption so that larger doses of lignocaine may be given.[2] Adrenaline in solution is rapidly degraded by oxidation, and solutions containing adrenaline therefore contain the antioxidant sodium metabisulphite at a concentration of between 0·05 and 0·1%. The constituents of some commonly used lignocaine preparations are shown in Table 1.

It has been suggested that the acidity of lignocaine preparations is the major cause for the burning sensation they produce, and the addition of sodium bicarbonate to both plain and adrenalized lignocaine has been advocated in an attempt to lessen discomfort.[4–8] The aim of this study was to investigate the characteristics of lignocaine preparations which may be responsible for the discomfort.

Methods

Subjects were recruited from patients attending the department of dermatology. Thirty-two patients (19 female) aged 17–85 years (mean 49 years) agreed to participate in the study. Each patient received a series of paired intradermal injections of different lignocaine preparations into normal skin on the volar aspect of one forearm using a 0·5-ml syringe with a 27-gauge needle. The pairs of injections were given in random order and in a double-blind manner. The skin was first swabbed with 70% isopropyl alcohol and allowed to dry. After the initial discomfort of the needle insertion had abated, 0·5 ml of each lignocaine preparation was injected slowly (over 15 s) by a single operator (C.L.) into the upper dermis, producing a weal. Each patient was asked to discount any discomfort caused by the initial needle insertion and to assess the discomfort caused by the injection of the lignocaine preparation. They were asked to state which member of each pair of injections was the less painful. The result was recorded as a preference for

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Table 1. Contents of commercial lignocaine preparations (per ml)

Preparation	Constituents
Lignocaine 0·5%	Lignocaine hydrochloride 5 mg
Lignocaine 2·0%	Lignocaine hydrochloride 20 mg
Lignocaine 0·5% with adrenaline 1:200,000 (Astra Pharmaceuticals) Multidose vial	Lignocaine hydrochloride 5 mg Adrenaline 5 μg Sodium metabisulphite 0·5 mg Methyl parabens 1 mg

(Note these aqueous preparations are made isotonic by the addition of sodium chloride.)

Table 2. Lignocaine preparations used

Solution	pH	Additives
A Lignocaine 0·5%	4·7	Nil
B Lignocaine 0·5% with adrenaline 1:200,000	4·6	Sodium metabisulphite 0·05 mg/ml
C Lignocaine 0·5% with sodium bicarbonate 8·4%*	7·4	Nil
D Lignocaine 2%	4·7	Nil
E Lignocaine 0·5% with sodium metabisulphite	3·1	Sodium metabisulphite 0·5 mg/ml
F Lignocaine 0·5% with adrenaline 1:200,000 (ASTRA Pharmaceuticals)	4·13	Sodium metabisulphite 0·5 mg/ml methylparabens 1 mg/ml

* 1 ml of 8·4% sodium bicarbonate added to 20 ml of 0·5% lignocaine.

Table 3. Pairs of lignocaine preparations administered

	Solution	To compare effect of
Pair 1 A vs. B	Lignocaine 0·5% Lignocaine 0·5% with adrenaline 1:200,000*	Adrenaline
Pair 2 A vs. C	Lignocaine 0·5% pH 4·7 Lignocaine 0·5% pH 7·4	pH
Pair 3 A vs. D	Lignocaine 0·5% Lignocaine 2·0%	Lignocaine concentration
Pair 4 A vs. E	Lignocaine 0·5% Lignocaine 0·5% with sodium metabisulphite 0·5 mg/ml	Sodium metabisulphite
Pair 5 E vs. F	Lignocaine 0·5% with sodium metabisulphite 0·5 mg/ml Lignocaine 0·5% with adrenaline 1:200,000†	Adrenaline (methylparabens)

* Contains sodium metabisulphite 0·05 mg/ml.

† Contains sodium metabisulphite 0·5 mg/ml, and methylparabens 1 mg/ml.

that preparation. If there was no difference in the discomfort experienced then no preference was recorded.

The lignocaine preparations used are shown in Table 2; their pH was measured using a Corning 120 pH meter. The pairs of injections given are shown in Table 3. The members of each pair differed by one major variable, and in this way it was possible to assess its contribution to the discomfort caused. All 32 subjects received Pairs 1–3 and 22 subjects received Pairs 1–5.

Results

The results of the assessments of the paired injections are shown in Tables 4 and 5. The results were analysed for statistical significance using McNemar's test. The effect of adrenaline on the degree of discomfort was investigated in Pair 1. Plain 0·5% lignocaine was found to be less painful than 0·5% lignocaine with adrenaline 1:200,000 by 23 (72%) of the subjects ($P<0{\cdot}001$). Solutions of adrenaline contain sodium metabisulphite as an antioxidant, and this was present in the adrenaline 1:10,000 that we used to make up solution B. However, the final concentration of sodium metabisulphite in solution B was only 0·05 mg/ml, 10 times less than that in commercial adrenalized lignocaine.

In Pair 2, the effect of the pH was assessed. Fourteen (44%) subjects found that neutral 0·5% lignocaine (pH 7·4) was less painful than standard 0·5% lignocaine (pH 4·7). Seven (22%) preferred the more acidic solution and 11 (34%) found no difference. In Pair 3, 0·5% lignocaine was found to be less painful than 2% lignocaine by all 32 subjects ($P<0{\cdot}001$), and 26 out of the 32 subjects tested said that 2% lignocaine was the most painful of all the injections they received.

In Pair 4, 18 (82%) of the 22 subjects tested found plain 0·5% lignocaine less painful than 0·5% lignocaine with sodium metabisulphite 0·5 mg/ml ($P<0{\cdot}001$). Two (9%) found no difference and a further two preferred the lignocaine with sodium metabisulphite.

In Pair 5, 18 (82%) of the 22 subjects tested found 0·5% lignocaine with sodium metabisulphite 0·5 mg/ml less painful than commercial 0·5% lignocaine with adrenaline 1:200,000 (although they both contain the same concentration of sodium metabisulphite). The additional discomfort caused by solution F was probably due to the presence of adrenaline, although we cannot exclude the possibility that the additional presence of methylparabens had a significant effect.

Table 4. Results of paired injections in 32 subjects

Lignocaine	Least painful (%)	No preference expressed (%)	*P*-value
Pair 1 (A vs. B)			
Lignocaine 0·5%	23 (72)	5 (16)	<0·001
Lignocaine 0·5% with adrenaline 1:200,000	4 (12)		
Pair 2 (A vs. C)			
Lignocaine 0·5% (pH 4·7)	7 (22)	11 (34)	NS
Lignocaine 0·5% (pH 7·4)	14 (44)		
Pair 3 (A vs. D)			
Lignocaine 0·5%	32 (100)	0 (0)	<0·001
Lignocaine 2·0%	0 (0)		

Table 5. Results of paired injections in 22 subjects

Lignocaine	Least painful (%)	No preference expressed (%)	*P*-value
Pair 4 (A vs. E)			
Lignocaine 0·5%	18 (82)	2 (9)	<0·001
Lignocaine 0·5% with sodium metabisulphite 0·5 mg/ml	2 (9)		
Pair 5 (A vs. F)			
Lignocaine 0·5% with sodium metabisulphite 0·5 mg/ml	18 (82)	3 (13·5)	<0·001
Lignocaine 0·5% with adrenaline 1:200,000*	1 (4·5)		

* Also contains sodium metabisulphite 0·5 mg/ml and methylparabens 1 mg/ml.

Discussion

Our results show that the concentration of lignocaine is a major factor influencing the discomfort caused by injection. All 32 subjects found that 2% lignocaine was more painful than 0·5% lignocaine. The addition of adrenaline to plain 0·5% lignocaine made injection more painful (Pair 1). This is consistent with the observation that 1% lignocaine with adrenaline 1:200,000 is more painful than plain 1% lignocaine.[9] The addition of sodium metabisulphite to plain 0·5% lignocaine also makes injection more painful. It therefore seems that the increased discomfort of adrenalized lignocaine is due to the presence of both adrenaline and sodium metabisulphite, and that their effects are additive.

McKay *et al.* found that freshly prepared solutions of lignocaine and adrenaline (where the adrenaline was added by the investigators themselves) were less painful than commercial preparations containing the same concentration of lignocaine and adrenaline.[4] They felt that the difference in the discomfort was due to the difference in pH of the two preparations. They also found that the discomfort caused by other local anaesthetics seemed related to their pH, with more acidic solutions causing a greater degree of discomfort. However, three of the five lignocaine solutions they compared also contained adrenaline. Two of these three were known by them to contain sodium metabisulphite, and it is likely that the third solution to which they added the adrenaline themselves also contained sodium metabisulphite. It would seem likely that the differences in discomfort they observed reflected the higher concentration of sodium metabisulphite found in commercial preparations.

Increasing the pH of both plain and adrenalized lignocaine by the addition of sodium bicarbonate has been found to reduce discomfort.[4–8] Whilst a greater proportion of our subjects preferred neutralized plain 0·5% lignocaine, this was not statistically significant. Clearly the influence of pH on the discomfort caused by local anaesthetics is less important than other factors, for example solution E at pH 3·1, was less painful than solution F at pH 4·13. Furthermore 1% procaine pH 4·3 is less painful than 1% lignocaine pH 6·3.[4]

Adequate anaesthesia is provided by 0·5% plain lignocaine for most minor surgical procedures. If more prolonged anaesthesia or the benefits of adrenaline are required then a technique similar to that suggested by Knowles[13] could be adopted. In this method, an initial injection of plain 0·5% lignocaine followed by injection of stronger lignocaine or adrenalized lignocaine into the already anaesthetized skin minimizes patient discomfort.

In conclusion, the results of our study show that the concentration of lignocaine, the presence of adrenaline, and the presence of sodium metabisulphite all affect the degree of discomfort associated with lignocaine preparations.

References

1 Grahame-Smith DG, Aronson JK. *Oxford Textboook of Clinical Pharmacology and Drug Therapy*. Oxford: Oxford Medical Publications, 1984; 552.

2 Grekin RC, Auletta MJ. Local anaesthesia in dermatologic surgery. *J Am Acad Dermatol* 1988; **19**: 599–614.

3 Arndt KA, Burton C, Noe JM. Minimising the pain of local anaesthesia. *Plast Reconstr Surg* 1983; **72**: 676–9.

4 McKay W, Morris R, Mushlin P. Sodium bicarbonate attenuates pain on skin infiltration with lidocaine, with or without epinephrine *Anaesth Analg* 1987; **66**: 572–4.

5 Stewart JH, Cole GW, Klein JA Neutralised lidocaine with epinephrine for local anaesthesia. *J Dermatol Surg Oncol* 1989; **15**: 1081–3.

6 Stewart JH, Chinn SE, Cole GW, Klein JA. Neutralised lidocaine with epinephrine for local anaesthesia II. *J Dermatol Surg Oncol* 1990; **16**: 842–5.

7 Korbon GA, Hurley DP, Williams GS. pH adjusted lidocaine does not 'sting'. *Anesthesiology* 1987; **66**: 855–6.

8 Christoph RA, Buchanan RN, Begalla K, Schwartz S. Pain reduction in local anesthetic administration through pH buffering *Ann Emerg Med* 1988; **17**: 117–20.

9 Morris RW, Whish DKM. A controlled trial of pain on skin infiltration with local anaesthetics. *Anaesth Intens Care* 1984; **12**: 113–4.

10 Winton GB. Anaesthesia for dermatologic surgery. *J Dermatol Surg Oncol* 1988; **14**: 41–54.

11 Wightman MA, Vaughan RW Comparison of compounds used for intradermal anesthesia. *Anesthesiology* 1976; **45**: 687–9.

12 Moore DC. The pH of local anaesthetic solutions. *Anesth Analg* 1981; **60**: 833–4.

13 Knowles WR. Minimising pain due to local anaesthesia *J Dermatol Surg Oncol* 1990, **16**: 489.

British Journal of Dermatology (1991) **125**, 456–459.

The treatment of 45 patients with cutaneous T-cell lymphoma with low doses of interferon-α2a and etretinate

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Accepted for publication 3 June 1991

Summary

Forty-five patients with cutaneous T-cell lymphomas (CTCL), 32 with mycosis fungoides (MF) and 13 with Sézary syndrome (SS), were treated with interferon-α2a (IFN-α2a) ($6–9 \times 10^6$ IU daily) for 3 months. Those responding to treatment were then treated with interferon-α alone ($6–9 \times 10^6$ IU three times weekly), and non-responders received a combination of etretinate (0·5 mg/kg/day) and IFN-α2a in similar concentrations. After 12 months of treatment, 28/45 patients (62·2%) were in complete or partial (> 50%) remission. Of these, 17 (60·7%) were receiving IFN-α alone and 11 the combined interferon–retinoid therapy. Of the patients with MF stage I and II, 20/25 were responders (12 receiving IFN-α alone and eight on combined therapy), whereas only 8/20 with stage IV or SS responded to treatment (five receiving IFN-α2a alone and three combined therapy). These results suggest that the association of etretinate with low-dose recombinant IFN-α2a is an effective means of treating epidermotropic CTCL, particularly in the early stages.

Retinoids are known to induce remission in some patients with cutaneous T-cell lymphomas (CTCL),[1,2] including Sézary syndrome (SS) and mycosis fungoides (MF). Several recent studies have demonstrated the efficacy of interferon-α in the treatment of CTCL, with a response in about 45–60% of patients.[3–5] However, only a few complete remissions were obtained and high doses of IFN-α2a ($18–30 \times 10^6$ IU/day) resulted in toxic effects, necessitating a reduction in the dose or discontinuing treatment. We have treated in a multicentre study, 45 patients with low-dose recombinant IFN-α2a ($6–9 \times 10^6$ IU/day) and etretinate was added after 3 months for those patients with a poor response.

Methods

Patients

In this multicentre study 45 patients were included. There were 25 men and 20 women and the mean age was 59 years (range 16–85 years). All had clinically active epidermotropic CTCL (32 MF and 13 SS). The patients were staged according to the Scandinavian MF study group[6] before interferon-α2a therapy, and 12 were stage I (four IA and eight IB). Thirteen were stage II (five IIA and eight IIB), seven were stage IVb and 13 were cases of SS. Most of the patients had previous treatment that included topical nitrogen mustard, PUVA therapy, retinoids, electron-beam irradiation and chemotherapy. None of the patients had any of these treatments in the 4 weeks before being included in the trial. In all the diagnosis was confirmed histologically. Patients were excluded who had hepatic, renal or cardiovascular dysfunction, other malignant disease, intercurrent infections and who were pregnant or lactating.

Investigations

The pretreatment assessment included a complete blood count, liver and renal function tests, chest X-ray, abdominal CT scan or ultrasound, a bone-marrow biopsy, and biopsies of palpable nodes and skin. During treatment repeat skin biopsies and counts for circulating Sézary cell were performed every 2 months; chest X-ray,

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abdominal CT scans or ultrasound after 6 and 12 months; and a repeat bone-marrow biopsy after 12 months of therapy.

Assessment of response

The response to treatment was evaluated clinically and histologically and assessed as those who had a complete or partial response who were responders, and those who had stable or progressive disease and were non-responders. A complete response was defined as the disappearance of all signs of disease activity for at least 4 weeks.

Treatment

The 45 patients received 6×10^6 IU interferon-α2a (Roche, France) daily during the first 2 months. After 2 months, responders received 6×10^6 IU IFN-α2a three times weekly for the next 10 months. Non-responders continued on IFN-α2a at a dose of 9×10^6 IU/day for another month. After 3 months, responders continued on 9×10^6 IU three times weekly for the next 9 months and for the non-responders etretinate (Tigason®) was added at a dosage of 0·5 mg/kg/day. After a further 2 months of treatment with IFN-α2a (6–9×10^6 IU/day) and etretinate, for those with progressive disease the therapy was discontinued (Fig. 1).

Results

Clinical response

The results are shown in Figure 2. After 3 months there were 19 responders (42·3%). After 5 months of treatment, 22 patients (48·8%) had a good response. At this time 10 stopped treatment; seven had progressive disease (three stage IV, four SS), two had side-effects (one stage I, one SS) and one patient with SS refused after 3 months to continue therapy. Of the remaining 35 patients, after 12 months 28 were responders (13 complete and 15 partial responders) and seven were non-responders. Of the 28 responders, 17 were treated with IFN-α alone (60·7%) and 11 had IFN-α and etretinate. Of the 45 patients, 10 who 'dropped out' after 5 months had to be considered as non-responders, thus there were 28 responders (62·2%) and 17 non-responders.

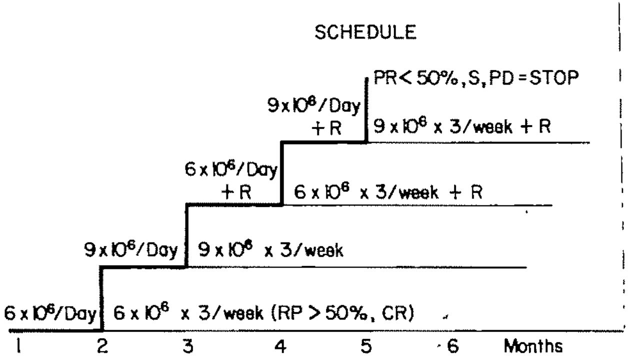

Figure 1. Treatment schedule. Interferon-α2a = 6–9×10^6 IU; R, retinoids (etretinate 1–2 mg/kg); CR, complete remission; PR, partial remission; SD, stable disease, PD, progressive disease.

The results according to clinical stage are shown in Table 1. Among the 25 patients with MF in stages I and II, after 12 months of treatment there were 20 responders (80%), 12 receiving IFN-α2a alone and eight receiving IFN-α2a and etretinate. Of the 20 patients with MF stage IVb or SS, only eight showed a response (40%) ($P<0{\cdot}001$); five receiving IFN-α2a alone (two MF, three SS) and three receiving IFN-α2a and etretinate (one MF, two SS). The combination of IFN-α2a and etretinate was given to 12 patients in stages I and II with good results in

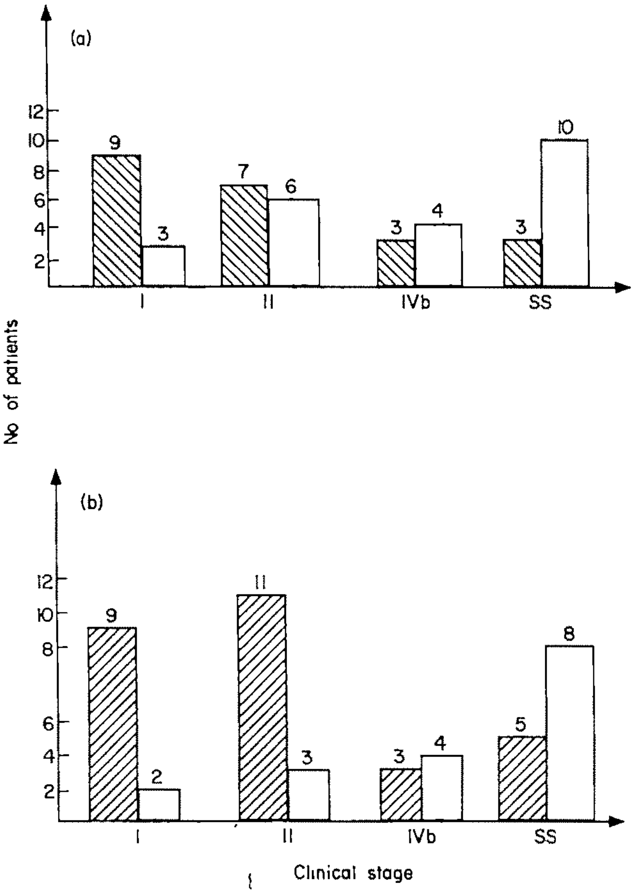

Figure 2. Good ▨ and poor ☐ responders after (a) 5 and (b) 12 months.

Table 1. Results according to clinical stage and treatment in 45 patients

	Early stage		Advanced stage	
	I	II	III	IV
INF alone, INF + ET				
Good results	20		8	
Poor results	5		12	

$P<0{\cdot}01$ χ^2 square $=7{\cdot}4$

	Early stage I, II		Stage III, IV	
	Good results	Poor results	Good results	Poor results
IFN	12	1	5	1
INF + ET	8	4	3	11

INF, interferon; ET, etretinate.

eight (67%), and to 14 patients in MF stage IVb or with SS with only three good results (21%) (one MF, two SS).

Two patients with stable disease after 3 months of IFN-α therapy were not given etretinate because of cardiac problems and IFN-α2a (9×10^6 IU three times a week) was continued for the next 9 months without noticeable change.

Side-effects

Sixty-nine per cent of patients had 'flu-like' symptoms, 44% had a fever, 60% skin dryness especially those receiving IFN-α2a and etretinate, 44% complained of nausea, 30% had diarrhoea and 30% depression. There was in 30% of the patients a neutropenia, 6% had a thrombocytopenia and in 24% there was an increase of the levels of the liver enzymes and a 30% increase in the levels of cholesterol and triglycerides. Treatment was stopped in two patients because in one there was severe thrombocytopenia after 4 months of IFN-α2a and in the other after 3 months because of a threefold increase in alkaline phosphatase. These patients were evaluated as having progressive disease.

Discussion

This study has demonstrated the beneficial effect of treatment with IFN-α in the early stages of MF either alone or in combination with etretinate. Those with the more advanced stages of MF responded well to treatment. It has been shown that CTCL respond in about 55% of cases to retinoids, but relapses occur when treatment is discontinued.[7] A few reports have shown beneficial results following treatment with a combination of etretinate and IFN-α in some patients with MF[8,9] and the efficacy of IFN-α alone has also been demonstrated.[10,11] In our study of a total of 45 patients the combination of low-dose IFN-α2a and etretinate was given to 26 who had the early and late stages of MF and who were poor responders. After 6 months good results occurred in 49% and after 12 months in 62%. Of those 28 patients with good results, 17 were treated with IFN-α alone (60·7%) and 11 with etretinate and interferon.

The results according to the clinical stage of the disease are of interest in that 80% of the patients with good results were in the early stages of MF. Furthermore, the combined regimen (etretinate with IFN-α2a) was more effective in patients in the early stages than those with the more advanced disease.

The response rate obtained in early stages of CTCL raises the question of the efficacy of treatment with IFN-α as compared to conventional therapies such as PUVA and topical nitrogen mustard. It would be of interest to determine the long-term effectiveness of treatment with IFN-α because with PUVA and treatment with topical nitrogen mustard only 30% of patients with CTCL in stage IB or IIB remain in complete remission after 5 years.[12,13] In our study, 14 patients in stage IB or IIB showing a complete response after 12 months of treatment have so far been followed up for 2 years. Eight patients are still in remission after discontinuing treatment for 8–12 months, and six patients have had a relapse 4–8 months after stopping treatment with IFN-α. However, resumption of treatment was again effective in five of these six patients.

In the patients with Sézary syndrome, only three had a good result with treatment with IFN-α alone and two others with combined therapy. Thus, most of the cases of Sézary syndrome were non-responders either to IFN-α alone or in combination with etretinate. However, it is well established that Sézary syndrome responds poorly to various treatments.

The mechanism of action of retinoids is complex. They appear to act primarily on the differentiation and proliferation of epithelial cells, but also could have an immunoregulatory effect on the mononuclear infiltrate in the skin.[14] IFN-α acts upon the proliferation of keratinocytes[15] and has an immunomodulatory effect, especially on macrophages and natural killer cells.[16]

This study of 45 patients with CTCL of different clinical stages confirms the efficacy of low-dose recombinant IFN-α2a in the treatment of CTCL and also combined interferon and retinoid therapy. The low doses of IFN-α2a were well tolerated, even after 12 months of treatment.

Acknowledgment

We thank Miss Arlette Bénardin for excellent technical assistance.

References

1 Claudy AL, Rouchouse B, Boucheron S, Le Petit JC. Treatment of cutaneous lymphoma with etretinate. *Br J Dermatol* 1983; 109: 49–56.

2 Kessler JF, Levine N, Meyskens FL *et al.* Treatment of cutaneous T cell lymphoma (mycosis fungoides) with 13 *cis*-retinoid acid *Lancet* 1983; i: 1345–7.

3 Bunn PA, Foon KA, Ihde DC *et al.* Recombinant leukocyte A interferon: an active agent in advanced cutaneous T cell lymphoma. *Ann Int Med* 1984; 101: 484–8.

4 Olsen EA, Rosen ST, Vollmer RT *et al.* Interferon alpha-2a in the treatment of cutaneous T cell lymphoma. *J Am Acad Dermatol* 1989; 20: 395–407.

5 Simoni R, Cavalier R, Coppola G *et al.* Recombinant leukocyte interferon α2a in the treatment of mycosis fungoides. *J Biol Regul Homeostatic Agents* 1987; 1: 93–9.

6 Molin L, Thomsen K, Volden G, Groth O. Photochemotherapy (PUVA) in the pretumant stage of mycosis fungoides: a report from the Scandinavian Mycosis Fungoides study group. *Acta Derm Venereol (Stockh)* 1980; 61: 47–51.

7 Mahrle G, Thiele B. Retinoids in cutaneous T cell lymphomas. *Dermatologica* 1987, 175 (Suppl. 1): 145–50.

8 Braathen LR, McFadden N. Successful treatment of mycosis fungoides with the combination of etretinate and human recombinant interferon alpha 2a. *J Derm Treatment* 1989; 1: 29–32.

9 Thestrup-Pedersen K, Hammer R, Kaltoft K *et al.* Treatment of mycosis fungoides with recombinant interferon α2a alone and in combination with etretinate. *Br J Dermatol* 1988; 118: 811–18.

10 Dreno B, Godefroy WY, Fleischmann M *et al* Low dose recombinant interferon α in the treatment of cutaneous T cell lymphomas. *Br J Dermatol* 1989; 121: 543–4.

11 Hagberg H, Juhlin L, Scheynius A, Tjernlund V. Low dosage alpha interferon treatment in patients with advanced cutaneous T cell lymphoma. *Eur J Haematol* 1988; 40: 31–4.

12 Honigsmann H, Brenner W, Rauschmeier W *et al.* Photochemotherapy for cutaneous T cell lymphoma. *J Am Acad Dermatol* 1984; 10: 238–45.

13 Vonderheid EC, Tan EJ, Kantor AF *et al.* Long-term efficacy, curative parental and carcinogenicity of topical mechlorethamine chemotherapy in cutaneous T cell lymphoma. *J Am Acad Dermatol* 1989; 20: 416–28.

14 Dicken CN, Connolly SM. Systemic retinoids in dermatology. *Mayo Clin Proc* 1982; 57: 51–7.

15 Nickoloff BJ, Basham TY, Merigan TC, Morhenn VB Antiproliferative effect of recombinant α and γ interferons on cultured human keratinocytes. *Lab Investigation* 1984; 51: 697–701.

16 Saiki I, Dunegan MA, Fann AV, Koff WC. Regulatory effects on macrophages of human recombinant interferon α. J Int Res 1986; 6: 603–11.

British Journal of Dermatology (1991) **125**, 460–462.

Necrolytic migratory erythema without glucagonoma: the role of dietary essential fatty acids

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Accepted for publication 3 June 1991

Summary We report a case of necrolytic migratory erythema without a glucagonoma and which responded to treatment with essential fatty acids and zinc.

Necrolytic migratory erythema (NME) is a rare skin condition usually associated with glucagonoma, an alpha-cell tumour of the pancreas.[1] It has also rarely been described in association with chronic liver disease, and in malabsorption with villous atrophy.[2] We report a case of NME in a man with cirrhosis and normal glucagon levels, who responded to treatment with essential fatty acid supplements and zinc.

Case report

A 52-year-old man was referred to the dermatology clinic in February 1989 complaining of a burning erythematous rash over the previous 6 months. The rash first appeared on his elbows and then became more generalized. There was no past medical history of note but he admitted to drinking 12 bottles of Guinness a day for the last 15 years and had noticed ankle swelling, increased girth, and intermittent jaundice over the previous 3 months. On examination there were signs of chronic liver disease, spider naevi, jaundice, ascites and peripheral oedema. There was tender hepatomegaly and a generalized erythematous rash with purpura over both lower legs.

The patient was admitted for further investigations but shortly after admission he had a large haematemesis which led to worsening of his hepatic failure. Endoscopy showed marked oesophagitis and oesophageal varices which were injected. Over the next 4 weeks he underwent three further endoscopies with sclerotherapy on two occasions, and was discharged on 5 April 1989. When he was seen again on 19 April the rash had reappeared as a gyrate scaly erythematous eruption on the feet, legs, groins and abdomen, with bullae present at the edges of the rash. Glossitis and angular stomatitis were also noted. The clinical appearance was typical for necrolytic migratory erythema (Fig. 1). A skin biopsy showed ortho- and parakeratosis, irregular acanthosis and areas of epidermal necrosis, in which the lower half of the epidermis was normal and the upper layers showed abrupt necrolysis. There was eosinophilia of the

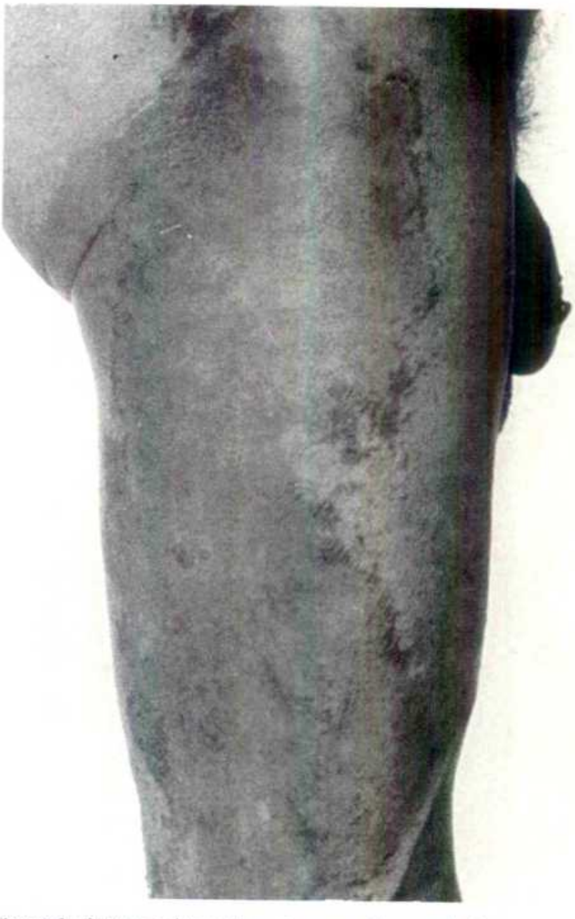

Figure 1. Gyrate scaly erythematous eruption on the leg.

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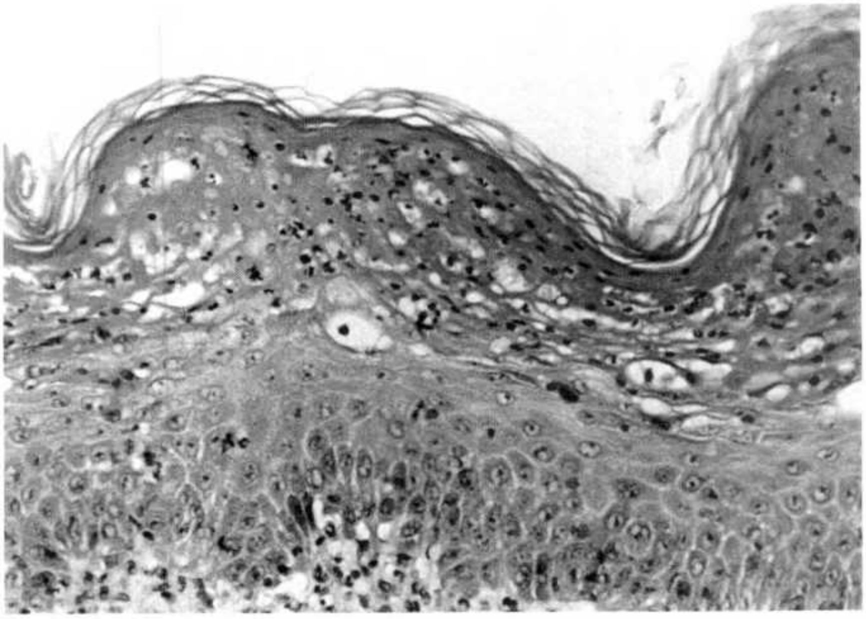

Figure 2. Necrolytic changes in the epidermis.

upper epidermal layers with spongiotic vesicles and neutrophil abscesses in the cornified layers. A mainly lymphocytic infiltrate was present in the papillary dermis. The appearance was characteristic of NME (Fig. 2).

The patient was thought to have the glucagonoma syndrome, but the glucagon level was normal at 12 pmol/l (NR < 50), and the serum amino-acid profile was normal. Serum zinc was significantly lowered at 8 μmol/l (NR 11·5–24·5). An ultrasound scan of the abdomen showed fatty change in the liver but no other abnormality, although the pancreas was partly obscured by gas. A CAT scan demonstrated normal liver, kidneys and pancreas.

Table 1. Analysis of plasma and red cell phospholipid fatty acids

	Plasma			Red cell phospholipid		
Fatty acid	Before treatment	After treatment	Control $n = 32$	Before treatment	After treatment	Control $n = 32$
20:3n-6	1·51	1·91	2·93	2·04	1·63	1·64
20:4n-6	5·70	6·40	11·11	13·83	13·78	18·15
20:5n-3	1·44	5·02	1·42	1·46	1·42	1·43
22:6n-3	1·74	4·45	4·34	5·49	5·63	6·32

On 31 May 1989 he was commenced on essential fatty acid supplements in the form of omega-3 triglycerides (Maxepa) 5 g daily and gamolenic acid (Epogam) eight capsules daily. Four weeks later there was a marked improvement in the rash although there were still some active lesions present in the flexures. At this stage zinc sulphate 200 mg t.d.s. was added to the treatment regime. Six weeks later his skin was clear, and remained so until November when Maxepa was withdrawn, resulting in a flare-up of the rash within a month. After reintroduction of the Maxepa the rash settled once more, and the patient remains well on this combination of drugs.

Plasma and red cell phospholipid fatty acids were analysed before and after treatment (Table 1).

Discussion

The glucagonoma syndrome was first described by Becker *et al.* in 1942.[1] The condition is caused by an alpha-cell tumour of the pancreas with elevated serum glucagon levels and almost all the patients have significant hypoaminoacidaemia.[2] The cutaneous features of the syndrome include angular stomatitis, cheilitis, glossitis, diffuse alopecia and brittle nails, as well as a necrolytic migratory erythema. Rare cases of NME have been reported where no tumour has been found, for example in association with cirrhosis[3] or malabsorption and villous atrophy.[4]

The cause of the skin changes in NME is unclear. Mallinson *et al.*[5] suggested that low amino-acid levels lead to depletion of epidermal protein and necrolysis. Intravenous amino acids have been used to treat several cases of NME with successful responses in five out of the six reported cases.[6] In our patient, however, the amino-acid profile was normal, and another case of NME in a patient with malabsorption who had normal amino acids has been recorded.[4] The similarities, both clinical and histological, between NME and zinc and niacin deficiencies have been noted.[7] Four cases have been published in which oral diiodohydroxyquinolone, a treatment for zinc deficiency, cleared or significantly improved the rash of NME,[4,5] and the possibility of a pathophysiological connection between NME, pellagra and zinc deficiency has been raised.[7] The patient we describe in this report had a low serum zinc level and cirrhosis and alcoholism are known to cause zinc deficiency by increasing urinary loss.[8]

The plasma and red cell membrane phospholipid fatty acids in our patient show marked abnormalities, with particularly low levels of six desaturated derivatives of linoleic acid in plasma, despite high or normal levels of linoleic acid itself. This suggests defective delta-6-desaturation of linoleic acid. The delta-6-desaturase enzyme is known to be inhibited by zinc deficiency or excess alcohol, as well as being dependent on the availability of nicotinamide-adenine dinucleotide (NAD).[9] The n-6 series of fatty acids are more prone to being affected by this interference than the n-3 series. It is of note that supplementation with the n-6 essential fatty acids (EFA) in the form of evening primrose oil (Epogam) has not succeeded in returning levels of dihomo-gamma linoleic acid (DGLA:20:3n-6) or arachidonic acid (AA:20:4n-6) to normal. Recent studies have suggested that for this to happen much larger doses may be required.[10] Supplementation with n-3 fatty acids however has resulted in an increase of eicosapentaenoic acid (EPA:20:5n-3) of up to nearly four times its normal level. Its longer chain derivative, docosahexaenoic acid (DHA:22:6n-3) has returned to normal after supplementation. It may be that the increase in the ratio of EPA to AA has resulted in a reduction in the synthesis of proinflammatory eicosanoids from arachidonic acid, reducing cutaneous inflammation. Alternatively, the return of DHA levels to normal may have had a membrane-stabilizing effect.

The response of our patient to a combination of EFA supplements and zinc sulphate supports the view that there may be a common metabolic pathway involved in the pathogenesis of NME and EFA deficiency.

References

1 Becker SW, Kahn D, Rothman S. Cutaneous manifestations of internal malignant tumours. *Arch Dermatol Syph* 1942; **45:** 1069–80.

2 Stacpoole PW. The glucagonoma syndrome, clinical features, diagnosis, and treatment. *Endocr Rev* 1981; **2:** 347–61.

3 Doyle JA, Schroeter AL, Rogers RS. Hyperglucagonaemia and necrolytic migratory erythema in cirrhosis—possible pseudoglucagonoma syndrome. *Br J Dermatol* 1979; **101:** 581–7.

4 Goodenberger DM, Lawley TJ, Strober W *et al.* NME without glucagonoma. Report of two cases. *Arch Dermatol* 1979; **115:** 1429–32.

5 Mallinson CN, Bloom SR, Warin AP *et al.* A glucagonoma syndrome. *Lancet* 1974; **ii:** 1–5.

6 Parker CM, Hanke CW, Madura JA, Liss EC. Glucagonoma syndrome: case report and literature review. *J Dermatol Surg Oncol* 1984; **10:** 884–9.

7 Miller SJ. Nutritional deficiency and the skin. *J Am Acad Dermatol* 1989; **21:** 1–30.

8 Neldner KH. Acrodermatitis eneropathica and other zinc deficiency disorders. In: *Dermatology in General Medicine* (Fitzpatrick TB *et al.*, eds). 3rd edn, Vol 2, 1987; 1616.

9 Brenner RR. Nutritional and hormonal factors influencing desaturation of essential fatty acids. *Prog Lipid Res* 1981; **20:** 41–7.

10 Abraham RDA, Riemersma RA, Oliver MF. Effect of evening primrose oil supplement on adipose tissue long chain polyunsaturated fat composition and serum lipids in human volunteers. *Aust NZ Med J* 1989; **19**(Suppl. 1): 560.

British Journal of Dermatology (1991) **125**, 463–465.

Psoriasis induced by interferon-α

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Accepted for publication 5 June 1991

Summary

Recombinant human interferon-α has been used in the treatment of several cancers, but there have been several reports that it may exacerbate psoriasis or trigger off its onset. We report four patients, three of whom first developed psoriasis and one who had an aggravation of the condition during treatment with interferon-α. Three of the patients had the carcinoid syndrome and one a renal carcinoma, and all were treated with interferon-α 2b or 2a (IFN-α2b, 2a) with doses ranging from $1{\cdot}5\times10^6$ U daily to 18×10^6 U three times weekly. In two of the patients there appeared to be a correlation between the severity of the psoriasis and the dosage of interferon.

Recombinant human interferon-α (IFN-α) has a well-documented anti-tumour effect[1–3] and has been used in the treatment of malignant metastatic carcinoid tumour and metastatic renal carcinoma.[4,5] Various cutaneous side-effects have been reported and these have included dryness and itching of the skin and a moderate amount of hair loss.[6] In 1986 three cases were reported of patients in whom psoriasis was aggravated or provoked during treatment with recombinant human IFN-α.[7] Also, the psoriasis in three patients was aggravated when IFN-α was given subcutaneously in the treatment of malignant melanoma.[8] Some patients in clinical trials of IFN-α have had an exacerbation of their psoriasis,[9,10] and this report describes four cases where treatment was followed either by an exacerbation or initial onset of this disorder.

Case reports

The four cases are summarized in Table 1. Cases 1, 2 and 3 were treated for the carcinoid syndrome with IFN-α2b (Intron A, Schering-Plough Co, Kenilworth, NJ, U.S.A.). Case 4 was a patient with renal carcinoma who was treated with IFN-α2a (Roferon, Hoffman-La Roche & Co, Basel, Switzerland).

Case 1

A 72-year-old man developed carcinoid syndrome in 1985 and was found to have metastases in the liver, omentum and small intestine. From October 1985 he was given subcutaneously IFN-α2b 5×10^6 U daily. Treatment was discontinued in October 1986 because of a lack of response by the tumours and an aggravation of his psoriasis. He had had psoriasis since 1943 and prior to treatment with interferon the lesions were limited to the elbows and the knees. Two weeks after the start of treatment with interferon he developed more generalized plaques and progressed in a few weeks to an erythroderma. The diagnosis of psoriasis was confirmed by a skin biopsy. Topical treatment and photochemotherapy (PUVA) produced only a temporary improvement and during the period December 1985 to October 1986 he was admitted to hospital four times. After treatment with interferon was stopped in October 1986 his psoriasis improved and after 2 weeks only involved the elbows and knees. He had no further treatment with interferon and his psoriasis remained mild. He died in 1989.

Case 2

A 56-year-old woman had carcinoid syndrome from 1984 and from May 1985 to October 1986 she was treated with subcutaneous injections of Intron A, receiving 5×10^6 U daily. Treatment was stopped because of severe psoriasis, which was thought to have been caused by the interferon. In February 1987 IFN-α was again given at a daily dose of 3×10^6 U daily because of progression of symptoms from her carcinoid syndrome. From June 1987 she was given $1{\cdot}5\times10^6$ U daily and by June 1990 remained well. This patient had no history of psoriasis before starting treatment with IFN-α, although her daughter had the condition. Her psoriasis first appeared after 17 months of treatment with lesions on the elbows and legs. When interferon therapy was

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Table 1. Four cases of psoriasis induced by IFN-α

Patient	Sex	Age (years)	Tumour	Skin lesions	Time interval before developing psoriasis (months)
Case 1	Male	72	Malignant carcinoid	Severe generalized psoriasis	0·5
Case 2	Female	56	Malignant carcinoid	Plaques on elbow and legs	17
Case 3	Male	59	Malignant carcinoid	Plaques on limbs and scalp	5
Case 4	Male	52	Metastatic renal carcinoma	Elbows, knees, scalp, trunk and groins	1

stopped in October 1986 the psoriasis cleared within 4 weeks and when interferon treatment was reintroduced, returned within 2 weeks. When the dose of interferon was reduced to $1{\cdot}5\times10^6$ U daily her psoriasis again improved.

Case 3

A 59-year-old man had symptoms of carcinoid syndrome from 1987 and a diagnosis of metastatic carcinoid tumour was made in April 1988 and metastases found in the liver. Treatment with subcutaneous injections of IFN-α2b (Intron A, 5×10^6 U daily) was given from April 1988 to October 1989. Treatment was stopped because of neurological symptoms that were thought to be side-effects from the interferon. This patient had no personal or family history of psoriasis and 5 months after the start of interferon therapy he developed erythematous hyperkeratotic plaques on the limbs and in the scalp. These were typical for psoriasis and the diagnosis was confirmed by the histology. The lesions of psoriasis were also present at the sites of injection with interferon and usually appeared a few days afterwards and cleared within a week. In February 1989, in a week without treatment with interferon his psoriasis cleared, but 1 week after reintroducing therapy his psoriasis recurred. This was also associated with pain in several joints in the legs. Therapy with interferon was discontinued in October 1989 when his psoriasis and joint pains cleared.

Case 4

A 52-year-old man had his right kidney removed in June 1988 for a renal carcinoma. Two months later he was found to have pulmonary metastases and he was started on treatment with IFN-α2a (Roferon). He was given 18×10^6 U three times a week subcutaneously and in addition prednisolone 20 mg daily. The steroids were gradually reduced and stopped after 3 months. He had no personal or family history of psoriasis. Four weeks after starting treatment with interferon he developed typical lesions of psoriasis on the elbows, knees, scalp, trunk and in the groins. In September 1989 treatment with interferon was stopped due to a lack of response by the tumours and his psoriasis then slowly improved. Because of skeletal metastases interferon therapy was again given in May 1990 with him having 18×10^6 U three times a week combined with prednisolone 10 mg daily. His psoriasis has remained stable.

Discussion

The variations in the severity of psoriasis in our four cases are in our opinion unlikely to be coincidental and are likely to be related to the changes in the therapy with IFN-α. The course of psoriasis in our patients could not be related to the growth or regression of their tumours.[11] Our observations together with previous reports[7,8] suggest that IFN-α has an aggravating effect in psoriasis. Of interest is that in Case 3, lesions of psoriasis appeared at the injection sites. Localized lesions have also been described occurring on warts treated with IFN-α.[12] Although systemic steroids may aggravate psoriasis, in Case 4 this developed early on and was unlikely to be related to the steroids and more likely to the interferon.

Interferons are produced by macrophages, fibroblasts and lymphocytes, and have a number of effects that could be related to the pathogenesis of psoriasis. They increase the lymphocytotoxic activity of natural killer lymphocytes[13] and are able to activate macrophages to increase phagocytosis.[14] In addition, interferons appear to induce keratinocytes to produce and release interleukin-1.[15] Immunosuppressive therapy is effective in psoriasis and therefore it could be expected that immunostimulation with interferon would have a reverse effect and exacerbate the condition. The cAMP/cGMP ratio is probably of significance in the pathogenesis of psoriasis[16] and the negative effect of β-adrenergic antagonists could be explained by the effect of these drugs on this ratio.[17] Lithium can provoke psoriasis and it has been shown that it inhibits adenylate cyclase and reduces the hormone-induced accumulation of cAMP in the cells.[18] Interferon also reduces the cAMP/cGMP ratio by increasing the intracellular level of cGMP.[19] Interferon-α has been shown to inhibit cell replication in tumours by interfering with mRNA translation[20] and also has an antiproliferative effect on human keratinocytes *in vitro*.[21] These effects could be expected to inhibit the development of psoriatic lesions in the skin, but this has not been found in clinical trials with the drug.[9,10]

We conclude that IFN-α can exacerbate and even provoke psoriasis in a predisposed individual. The mechanism by which this occurs is as yet unknown.

References

1 Spiegel RJ. Intron A (interferon alfa-2b). clinical overview and future directions. *Semin Oncol* 1986; **13** (Suppl 2): 89–101.

2 Hersey P. The evolving role of alpha interferon in the treatment of malignancies. *Aust NZ J Med* 1986, **16**: 425–37.

3 Kirkwood JM, Ernstoff MS. Interferons in the treatment of human cancer. *J Clin Oncol* 1984; **2**: 336–52.

4 Hanssen LE, Schrumpf E, Kolbenstvedt AN *et al* Treatment of malignant metastatic midgut carcinoid tumours with recombinant human alpha 2b interferon with or without prior hepatic artery embolization. *Scand J Gastroenterol* 1989; **24**: 787–95.

5 Edsmyr F, Esposti PL, Andersson L *et al* Interferon therapy in disseminated renal cell carcinoma *Radiother Oncol* 1985, **4**: 21–6

6 Feyeux C, Rybojad M, Puissant A. Interferon in dermatology. *Nouv Dermatol* 1987; **6** (Suppl. 4): 471–4.

7 Quesada JR, Gutterman JU. Psoriasis and alpha-interferon. *Lancet* 1986, **i**: 1466–8.

8 Hartmann F, von Wussow P, Deicher H. Psoriasis-Exazerbation bei Therapie mit alpha-Interferon. *Dtsch Med Wschr* 1989; **114**: 96–8.

9 Neumann R, Pohl-Markl H, Aberer E. Parenteral interferon-alpha treatment of psoriasis *Dermatologica* 1987; **175**: 23–8.

10 Harrison PV, Peat MJ. Effect of interferon on psoriasis. *Lancet* 1986; **ii**: 457–8.

11 Schadijew HK, Kalamkaryan AA. The course of psoriasis in cancer patients. *Br J Dermatol* 1983; **109**: 365

12 Shiohara T, Kobayashi M, Abe K, Nagashima M. Psoriasis occurring predominantly on warts. *Arch Dermatol* 1988; **124**: 1816–21.

13 Ho M. Recent advances in the study of interferon. *Pharmacol Rev* 1982; **34**: 119–29.

14 Stiehm ER, Kronenberg LH, Rosenblatt HM *et al.* Interferon immunobiology and clinical significance. *Ann Intern Med* 1982; **96**: 80–93.

15 Luger TA, Sztein MB, Schmidt JA *et al.* Properties of murine and human epidermal cell-derived thymocyte-activating factor. *Fed Proc* 1983; **42**: 2772–6.

16 Voorhees JJ, Marcelo CL, Duell EA. Cyclic AMP, cyclic GMP and glucocorticoids as potential metabolic regulators of epidermal proliferation and differentiation *J Invest Dermatol* 1975, **65**: 179–90.

17 Felix RH, Ive FA, Dahl MGC. Cutaneous and ocular reactions to practolol. *Br Med J* 1974; **4**: 321–4

18 Forn J. Lithium and cyclic AMP. In: *Lithium Research and Therapy*. (Johnson FN, ed). New York: Academic Press, Inc , 1975: 485–95

19 Tovey MG. Interferon and cyclic nucleotids. In. *Interferon* (Gresser I, ed). London: Academic Press, 1982; 23–42.

20 Yabrov AA. Interferon: anticancer agent having multifarious activity. *Prog Clin Cancer* 1982; **9**: 99–145.

21 Nickoloff BJ, Basham TY, Merigan TC *et al* Antiproliferative effects of recombinant alpha and gamma interferons on cultured human keratinocytes. *Lab Invest* 1984; **51**: 697–701.

British Journal of Dermatology (1991) **125**, 466–468.

Neurilemmomatosis

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Accepted for publication 29 May 1991

Summary A case is reported of a 13-year-old boy with multiple intradermal neurilemmomas, a cerebello-pontine meningioma and multiple spinal-cord tumours.

Neurilemmomas are benign nerve-sheath tumours which consist of Schwann cells and almost always occur as solitary lesions. They are usually subcutaneous tumours with a predilection for the head and neck and extremities, or they can occur as deeply situated tumours in the posterior mediastinum and in the retroperitoneum. Rarely the tumours are multiple and located intradermally. In 1984, Shishiba *et al.*[1] described four cases with multiple intradermal neurilemmomas, with tumours of the central nervous system and with various neurological defects. In the absence of features for neurofibromatosis, these cases were described under the title of neurilemmomatosis. Since their report several further cases of neurilemmomatosis have been described.[2–4] We present a case of neurilemmomatosis with multiple intradermal neurilemmomas and multiple tumours of the central nervous system.

Case report

A 13-year-old boy was referred with multiple tender skin tumours that had been present for 5 years and which were gradually increasing in size and number. There was no family history of neurofibromatosis or of any neurological disorders. There was no past history of epilepsy and he was not mentally retarded. Over several years he had become blind in the left eye, and there were disturbances of his gait, a left-sided hypacusis and hoarseness of his voice.

On examination there were multiple firm and slightly elevated pigmented plaques or flesh-coloured nodules that measured 1–2 cm in diameter, scattered on the face, trunk and limbs. They were attached to the skin but not to the underlying tissues. In addition to these dermal tumours there were large subcutaneous nodules in both axillae. There was some atrophy of the muscles of the limbs. Of note, there were no café-au-lait macules present and there was no freckling in the axillae. No Lisch nodules were seen although there were cataracts and a persistent primary vitreous of the left eye with nystagmus. An audiogram revealed that there was a perceptive deafness of the left ear. He was hoarse with

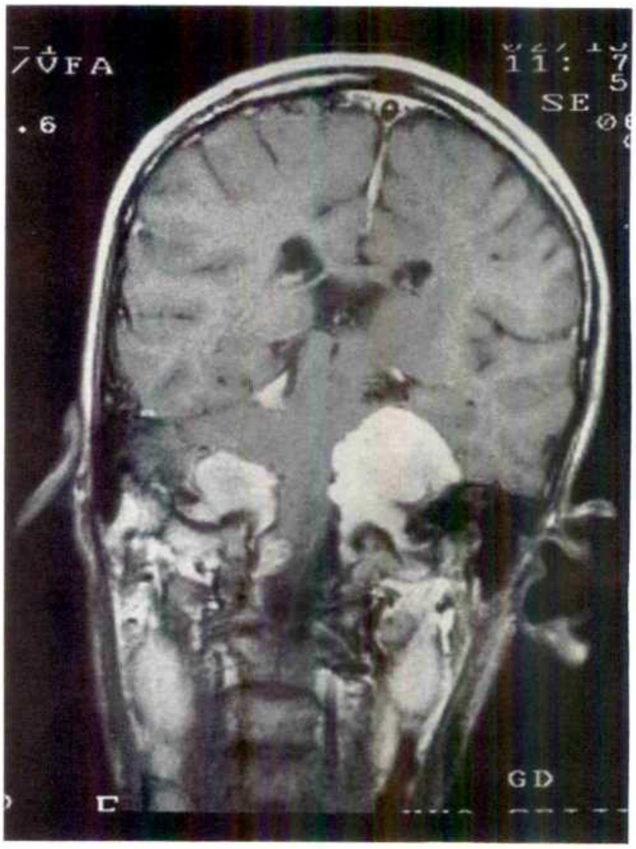

Figure 1. Magnetic resonance imaging showing bilateral cerebello-pontine angle tumours.

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paresis of the vocal cords due to bilateral paralysis of the recurrent laryngeal nerves.

Magnetic resonance imaging showed large shadows on both sides in the cerebello-pontine regions of the brain (Fig. 1) and multiple spinal tumours that were located at C_{3-6}, T_{7-9} and L_2–S_3. There were several small calcified nodules in the third and lateral ventricles that were interpreted as subependymomas. Other laboratory investigations were all normal and an X-ray of the chest showed no significant abnormality.

Biopsies taken from a lesion on the upper lip and on the chest showed plexiform tumours in the dermis that consisted of compact elongated spindle cells (Fig. 2) that appeared to be encapsulated by a thin layer of connective tissue. These cells showed nuclear pallisading and abundant Verocay bodies (Fig. 3). The histology of a subcutaneous tumour in the left axilla showed the characteristic features of a neurilemmoma. The tumour at the left cerebello-pontine region showed the histology of a meningotheliomatous meningioma with psammoma bodies.

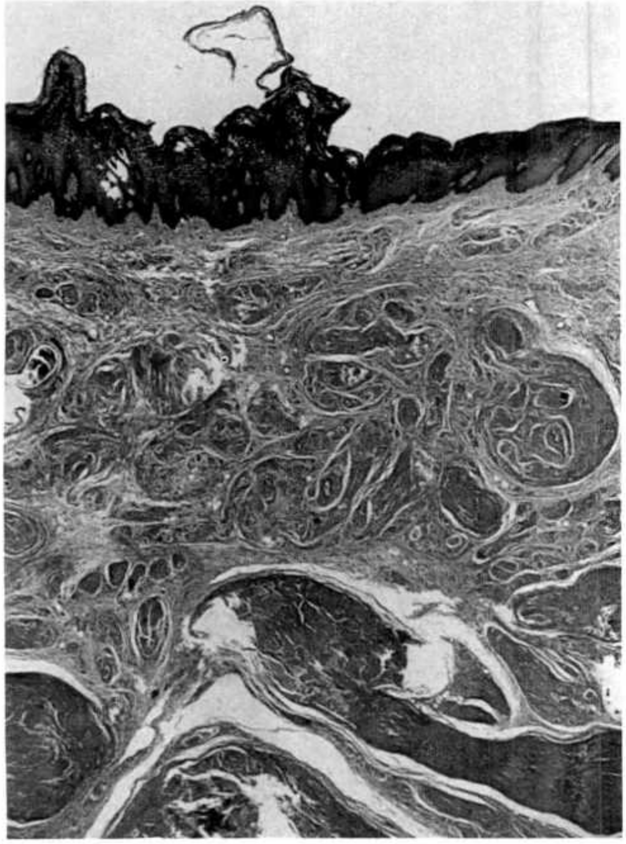

Figure 2. Tumour of the lip showing plexiform arrangement of interwoven cells in the dermis (haematoxylin and eosin, × 25).

Discussion

Although multiple subcutaneous neurilemmomas of one extremity without tumours of the central nervous system have been reported,[5] these tumours generally occur as solitary subcutaneous lesions. However, several

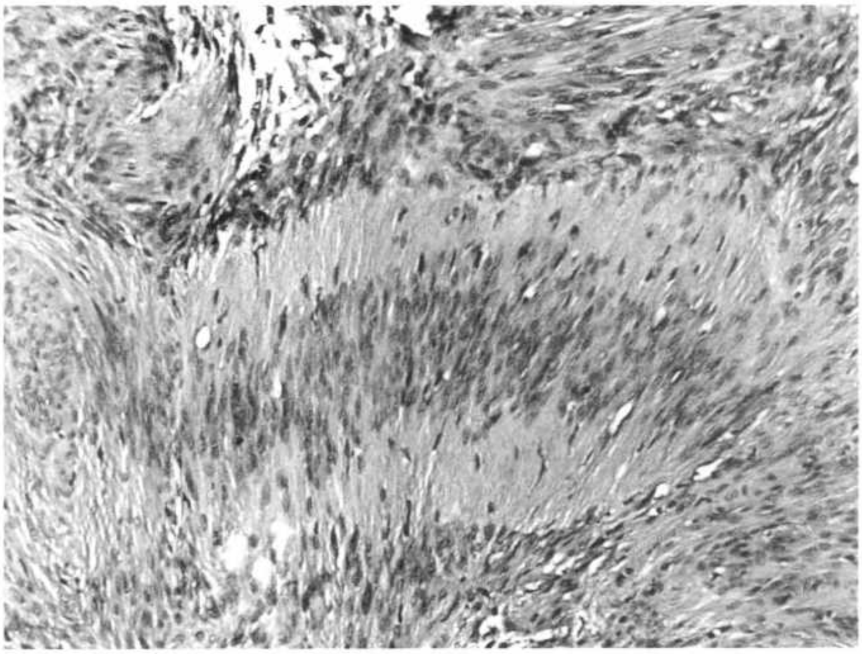

Figure 3. Higher magnification showing pallisading of the nuclei and Verocay bodies characteristic for neurilemmoma (haematoxylin and eosin, × 250).

cases have been reported in Japan who have had multiple intradermal neurilemmomas that were associated with tumours of the central nervous system and various neurological defects and have been referred to as 'multiple neurilemmomas' or 'neurinomatosis'.[1] However, it was not until after the report in 1984[1] that this condition was more widely recognized and distinguished from that of neurofibromatosis. The tumours of the central nervous system are usually neurilemmomas but sometimes gliomas, astrocytomas or meningiomas.

Our patient had multiple intradermal neurilemmomas, multiple tumours of the central nervous system and various neurological abnormalities such as hypacusia, disturbance of his gait and weakness and atrophy of the muscles of the limbs. His hypacusia and hoarseness could be attributed to the presence of the cerebello-pontine tumours, and the disturbance of his gait and the muscle atrophy of the limbs to the multiple tumours of the spinal cord. Because our patient had both cutaneous tumours and tumours of the central nervous system, a diagnosis of neurofibromatosis was considered but the histology of the tumours were characteristic for neurilemmomas and he had no other features of von Recklinghausen's disease (neurofibromatosis type 1).[6] Also, our case could be distinguished from neurofibromatosis type 2[6] where, although café-au-lait macules and cutaneous neurofibromas may be inconspicuous, the histology of thé left cerebello-pontine angle tumour was not that of a neurofibroma but a meningioma. Our patient also had various ophthalmological problems, and congenital cataracts have been reported in neurilemmomatosis and about 10% of the cases have an amblyopia or other visual disturbances.[1] The cutaneous lesions of neurilemmomatosis may be present at birth or develop in early life and precede the neurological abnormalities that usually develop in early adulthood. Dermatologists should be aware that the multiple cutaneous neurilemmomas may be a marker for central-nervous-system tumours and the appropriate neurological investigations undertaken.

References

1 Shishiba T, Niimura M, Ohtsuka F *et al.* Multiple cutaneous neurilemmomas as a skin manifestation of neurilemmomatosis *J Am Acad Dermatol* 1984; 10: 744–54.
2 Prevoo R, Starink TM. Multiple cutaneous neurilemmomas with abnormalities of the eyes and congenital rib deformities. *J Am Acad Dermatol* 1987; 17: 1054–5.
3 Purcell SM, Dixon SL. Schwannomatosis: An unusual variant of neurofibromatosis or a distinct clinical entity? *Arch Dermatol* 1989; 125: 390–3.
4 Rongioletti F, Drago F, Rebora A. Multiple cutaneous plexiform Schwannomas with tumors of the central nervous system. *Arch Dermatol* 1989; 125: 431–2.
5 Lewis RC, Nannini LH, Cocke WM Jr. Multifocal neurilemmomas of median and ulnar nerves of the same extremity—Case report. *J Hand Surg* 1981; 6: 406–8.
6 Riccardi VM. Neurofibromatosis heterogeneity. *J Am Acad Dermatol* 1984; 10: 518–19.

British Journal of Dermatology (1991) **125**, 469–471.

Ichthyosis bullosa of Siemens responds well to low-dosage oral retinoids

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Accepted for publication 10 June 1991

Summary Two patients with ichthyosis bullosa of Siemens (IBS) and one patient with bullous ichthyosiform erythroderma of Brocq (BIE) were treated with etretinate. Two additional patients with IBS received acitretin. All the patients had a marked improvement when on retinoids and the maintenance dose required was for IBS 10–25 mg per day. The patient with BIE was on a maintenance dose of 40–60 mg per day.

Bullous ichthyosiform erythroderma is a relatively rare but severe and incapacitating type of congenital ichthyosis. This type of epidermolytic hyperkeratosis has been described by Brocq[1] and further delineated by Lapière.[2] However, in 1937 Siemens distinguished a particular type of congenital bullous ichthyosis without erythroderma.[3] Over the years this type of ichthyosis has been described as having a relatively mild involvement of the skin and the epidermolytic hyperkeratosis has been restricted to the upper part of the epidermis.[4,5]

Aromatic retinoids have been effective in the treatment of various forms of congenital ichthyosis,[6–8] but the treatment of bullous ichthyosis erythroderma of Brocq with oral retinoids has been difficult.[9–12] Although a marked reduction of the hyperkeratosis can be achieved, exacerbation of the blistering is a serious complication and often limits the dose of the drug. As yet there have been no reports of the use of retinoids in the treatment of ichthyosis bullosa of Siemens (IBS). This present study was to compare the responses of these two types of bullous ichthyosis to therapy with oral retinoids.

Case reports

The clinical and histopathological features of Cases 1 to 4 have been previously described.[5] All the patients were monitored at 4-weekly intervals while on treatment.

Case 1

A 27-year-old woman weighing 63 kg had superficial blistering of the skin on the extensor surfaces of the arms and legs in addition to ichthyosis (Fig. 1a). Her father (Case 3), uncle (Case 4), sisters and son were also affected. At no time had she an erythroderma. A skin biopsy showed mild acanthokeratolysis of the epidermis restricted to the region of the stratum granulosum and upper part of the stratum spinosum.

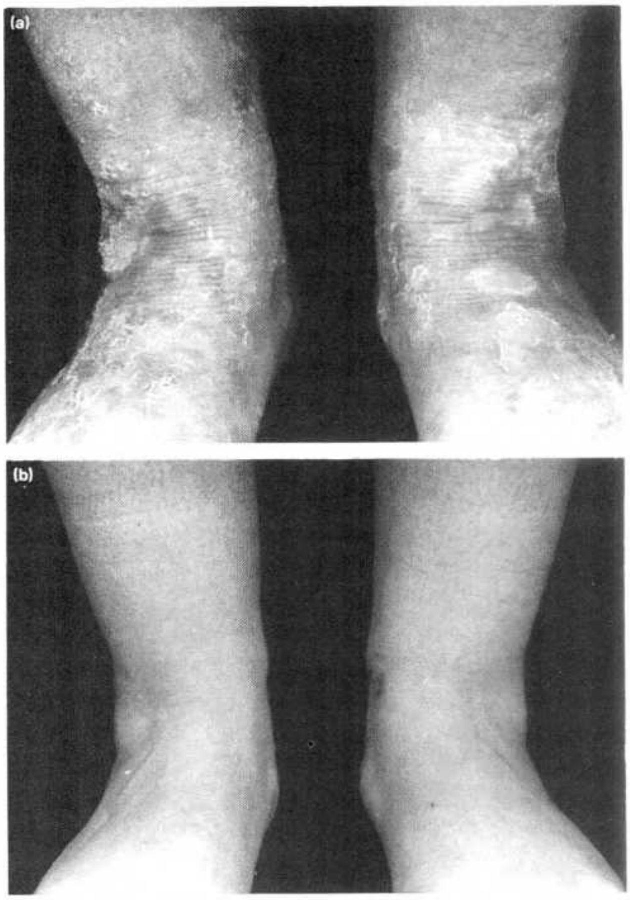

Figure 1. Ichthyosis bullosa of Siemens: (a) before treatment, (b) after 16 weeks of treatment with acitretin (25 mg/day).

The patient was given acitretin in a dosage of 35 mg daily and after 4 weeks there was marked reduction of the scaling that was virtually absent after 8 weeks of therapy. The dose was reduced after 12 weeks of treatment to 30 mg and further reduced after 16 weeks to 25 mg (Fig. 1b). The dosage of acitretin was again reduced at 20 weeks because of some hair loss and slight elevation of the liver transaminases. A further reduction of the dose was at 24 weeks when she was on 10 and 20 mg on alternate days. During the follow-up period of 95 weeks no blistering of the skin had occurred.

Case 2

A 21-year-old woman weighing 77 kg presented with the same degree of involvement as seen in Case 1. The patient was given acitretin in a dosage of 35 mg daily and the hyperkeratosis diminished and was virtually absent after 8 weeks of therapy. The dosage was reduced to 30 mg and an excellent response was maintained from 13 weeks with a dosage of 25 mg. During the follow-up period of 22 months no blistering of the skin has occurred.

Case 3

The 51-year-old father of Cases 1 and 2 and weighing 77 kg had suffered from a scaly skin since birth. From the age of 15 he also had chronic relapsing pustular eruptions and a biopsy showed a subcorneal blister that was filled with numerous polymorphonuclear leucocytes. He was given etretinate in a dosage of 35 mg daily and after 4 weeks the skin had improved and there was no further pustulation. After 8 weeks of treatment the hyperkeratosis was hardly noticeable and the dosage was further reduced to 25 mg per day. In the 22nd week the dosage was further reduced to 25 mg on alternate days and by the 28th week to 10 mg daily. There was mild scaling of the arms but over the observation period of 14 months there was no blistering or pustulation of the skin.

Case 4

The 40-year-old uncle of Case 1 weighing 65 kg had the same disorder with relapsing pustular eruptions and was given etretinate in a dosage of 35 mg daily. After 4 weeks of treatment the hyperkeratosis and pustulation had resolved and the dosage of the retinoid was reduced to 25 mg daily. A further reduction to 10 mg daily resulted in an exacerbation with pustulation and scaling. Over a follow-up period of 12 months a good response was maintained with him having 10 and 20 mg of etretinate on alternate days.

Case 5

A 15-year-old male weighing 67 kg had severe hyperkeratosis (Fig. 2a) that covered the entire surface of the body apart from the face, palms and soles. The condition

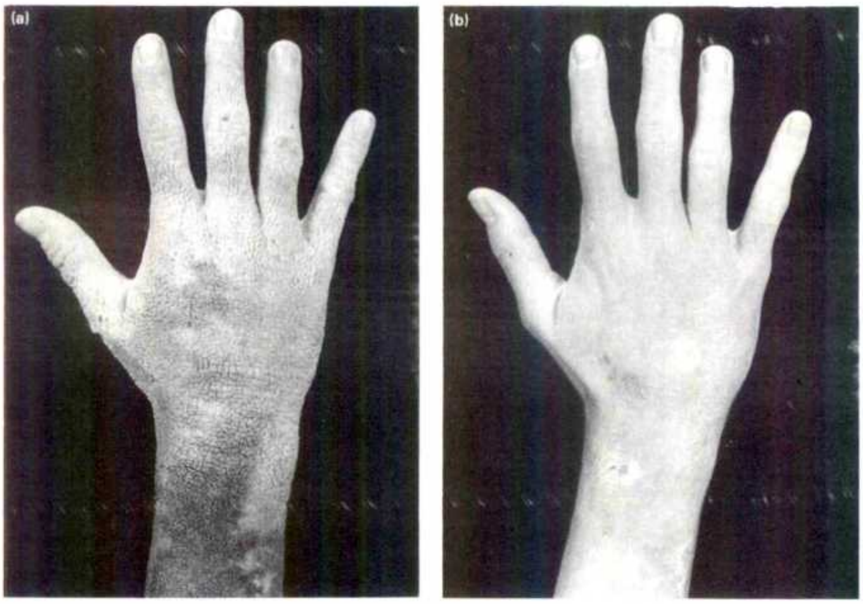

Figure 2. Bullous ichthyosiform erythroderma of Brocq: (a) before treatment, (b) after 10 weeks of treatment with etretinate (50 mg/day). Note the appearance of some blisters.

had been present since birth, but because the patient was an orphan it was uncertain about his first year of life and a family history was not available. A skin biopsy from the left forearm showed features of an epidermolytic hyperkeratosis that involved the entire epidermis.

He was treated with etretinate in a dosage of 75 mg daily and within 4 weeks there was a marked improvement. However, a skin biopsy showed even more pronounced histological features despite the clinical improvement. During the subsequent 6 months he was treated with etretinate in a dosage of between 40–60 mg daily and apart from slight recurrence of mild hyperkeratosis with bulla formation he was otherwise satisfactory (Fig. 2b).

Discussion

Ichthyosis bullosa of Siemens is a relatively mild condition as compared to bullous ichthyosiform erythroderma. However, the extent of the lesions and the discomfort caused by scaling and pustulation in our cases justified the administration of oral retinoids. As yet there are no other reports on the response of this disorder to systemic retinoids. None of our four patients showed any sign of blistering of the skin during the follow-up period of 12–95 weeks. The maintenance dose was much lower than that required for bullous ichthyosiform erythroderma.

The efficacy of etretinate in the treatment of BIE is shown in our Case 5, though the maintenance dose of the drug was high at 40–60 mg daily. A maximum dose of retinoids is limited by induction of blisters in this disorder[9] and it has also been reported that the blister formation was not inhibited by therapy with etretinate[11] as we found in our case.

Acknowledgments

We thank Dr J.M.Geiger and Professor B.M.Czarnetski for helpful discussions and Hoffman-La Roche for providing acitretin.

References

1 Brocq L. Erythrodermie congénitale ichthyosiforme avec hyperépidermotrophie. *Ann Derm Syph* 1902; **3:** 1–31.

2 Lapière S. Les génodermatoses hyperkératosiques de type bulleux. *Ann Dermatol Venereol* 1953; **80:** 597–614

3 Siemens HW. Dichtung und Wahrheit über die 'Ichthyosis bullosa', mit Bemerkungen zur Systematik der Epidermolysen. *Arch Dermatol Syph (Berl)* 1937; **175:** 590–608

4 Traupe H, Kolde G, Hamm H, Happle R. Ichthyosis bullosa of Siemens: a unique type of epidermolytic hyperkeratosis. *J Am Acad Dermatol* 1986; **14:** 1000–5.

5 Steijlen PM, Perret CM, Schuurmans Stekhoven JH *et al.* Ichthyosis bullosa of Siemens: Further delineation of the phenotype. *Arch Dermatol Res* 1990; **282:** 1–5

6 Happle R, Van de Kerkhof PCM, Traupe H. Retinoids in disorders of keratinization. Their use in adults *Dermatologica* 1987; **175** (Suppl. 1): 107–24.

7 Schimpf A Zur systemischen Anwendung eines aromatischen Vitamin-A-Säure-Derivates (RO 10-9359) bei Psoriasis und Keratosen. *Z Hautkr* 1976, **51:** 265–74.

8 Marks R, Finlay AY, Holt PJA. Severe disorders of keratinization. effects of treatment with Tigason (etretinate). *Br J Dermatol* 1981; **104:** 667–73.

9 Blanchet-Bardon C, Anton-Lamprecht I, Schnyder UW Erythrodermie congénitale ichthyosiforme bulleuse. Contrôle ultrastructural du traitement par l'éther éthylique d'un dérivé aromatique de l'acide rétinoique. *Ann Dermatol Venereol* 1977; **104:** 648–53.

10 Schnijder UW, Vogel A Zur behandlung der Erythrodermia congenitalis ichthyosiformis bullosa mit aromatischen Retinoiden. *Akt Dermatol* 1980; **6:** 133–6.

11 Dinet Y, Achten G, Wanet J *et al.* Traitement oral de grands états ichthyosiformes par le rétinoïde éthylester. *Ann Dermatol Venereol* 1978, **105:** 465–8.

12 Atherton DJ, Wells RS Etretinate in the ichthyosiform erythrodermas. In: *Retinoid Therapy. A Review of Clinical Laboratory Research* (Cunliffe WJ, Miller AJ, eds) Lancaster: MTP Press, 1984, 63–71

British Journal of Dermatology (1991) 125, 472–474.

Pulmonary capillary leak syndrome complicating generalized pustular psoriasis: possible role of cytokines

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Accepted for publication 18 June 1991

Summary Two cases are reported of generalized pustular psoriasis complicated by profound alterations in pulmonary capillary permeability. Several features suggest the involvement of cytokines in the pathogenesis of this condition.

Cardiorespiratory failure is reported as the commonest cause of death in patients with erythrodermic dermatoses, including pustular psoriasis where the mortality approaches 70%.[1] We report two cases of severe generalized pustular psoriasis complicated by profoundly increased pulmonary capillary permeability, a condition referred to as capillary leak syndrome (CLS). We propose that pulmonary inflammation coincident with extensive cutaneous disease might result from the action of epidermal cell-derived inflammatory mediators on the pulmonary vasculature.

Case reports

Patient 1

A 23-year-old previously fit man presented with a pustular eruption on his face and trunk following a 'flu-like illness of 3 days' duration. There was no personal history nor family history of psoriasis or other skin disorders.

On examination, he appeared unwell and pyrexial (38·5°C). An extensive eruption typical of pustular psoriasis was present on his scalp, face and trunk with psoriasiform pitting of all finger- and toenails which he had noted for approximately 1 year prior to presentation. Cardiorespiratory examination was normal.

Biochemical and haematological parameters were normal except for a neutrophil leucocytosis of $52{\cdot}6 \times 10^9$/l (normal range $3{\cdot}5$–$11{\cdot}5 \times 10^9$/l). Bacterial cultures of blood, urine and sputum were repeatedly negative and viral titres, including human immunodeficiency virus and cytomegalovirus, were also negative. A chest radiograph was normal.

Over the next 2 days, the eruption became progressively more extensive and necrotic (Fig. 1) with widespread cutaneous oedema and pustulation, sparing only the palms and soles. Methotrexate (5 mg orally for 3 days) and flucloxacillin were started on day 3 of hospital admission. On day 5 the patient became increasingly tachypnoeic and subsequently rapidly hypoxic. A chest radiograph revealed extensive bilateral alveolar shadowing consistent with profound pulmonary oedema (Fig. 2). Despite mechanical ventilation, the patient had an asystolic cardiac arrest and died 4h after initial onset of respiratory symptoms. Post-mortem examination revealed extensive interstitial pulmonary oedema with evidence of hyaline membrane formation, diagnostic of capillary leak syndrome. There was no evidence of inclusion bodies nor eosinophilia in the pulmonary sections to suggest either a drug-induced or viral aetiology.

Patient 2

A 64-year-old female with a 2-year history of plaque psoriasis developed a pustular eruption on the trunk following a walking holiday in New Zealand. Eighteen months earlier during admission for a shoulder replacement for osteoarthritis, radiological evidence of early pulmonary fibrosis was detected, although she remained entirely asymptomatic.

On initial examination, a pyrexia (37·8°C) was recorded and an eruption typical of generalized pustular psoriasis was present on the trunk and limbs. A chest radiograph at that time revealed no deterioration in her pulmonary fibrosis.

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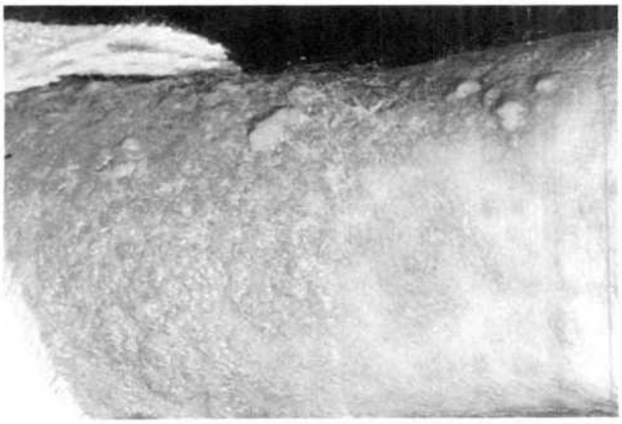

Figure 1. Forearm of Patient 1 demonstrating marked oedema and extensive pustulation, coalescing in areas to form blisters.

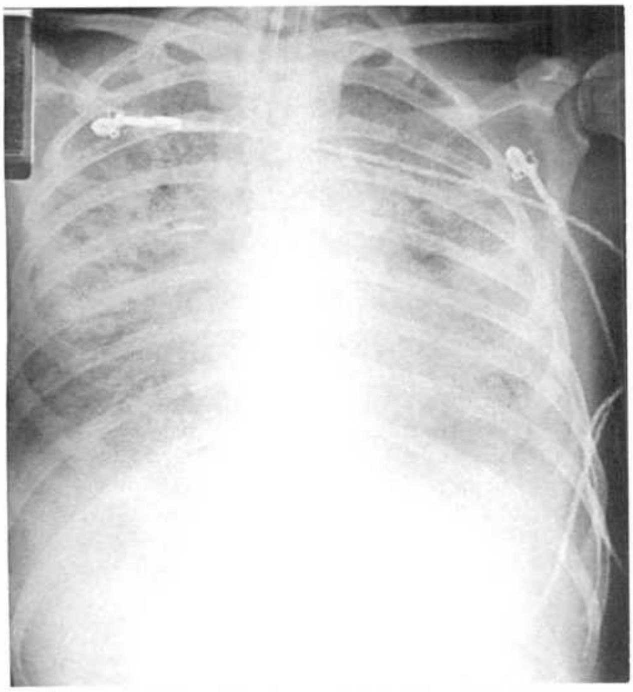

Figure 2. Chest radiograph of Patient 1 performed shortly before death demonstrating bilateral interstitial oedema.

Laboratory investigations revealed a neutrophil leucocytosis of 22×10^9/l and normal renal and liver biochemistry. Azathioprine (100 mg daily) and etretinate (40 mg daily) were started with initial improvement. However, 10 days later a relapse of her pustular psoriasis coincided with an episode of acute breathlessness. A chest radiograph was suggestive of pulmonary oedema, but this condition proved unresponsive to antibiotic and diuretic therapy. Echocardiography confirmed normal left ventricular function and a clinical diagnosis of pulmonary capillary leak syndrome was made. Improvement in respiratory function coincided with subsequent control of the pustular psoriasis. On four further occasions, acute episodes of breathlessness and pulmonary oedema occurred coincident with relapses of her pustular psoriasis.

Twelve weeks after initial presentation the patient was readmitted with respiratory failure from which she died 2 weeks later. Post-mortem examination confirmed a diagnosis of end-stage pulmonary fibrosis.

Discussion

Pulmonary capillary leak syndrome (CLS) is an inflammatory lung condition characterized by a profound increase in pulmonary capillary permeability.[2] It is a potentially life-threatening complication of many conditions, including severe sepsis, amniotic fluid embolus, major trauma and cutaneous burns. A clinical diagnosis of CLS is made when severe hypoxia associated with radiological evidence of pulmonary oedema occurs in the presence of normal left ventricular function. Although definitive diagnosis requires pulmonary angiography, in practice the invasive nature of this procedure precludes its routine use.

Both clinical and post-mortem examination suggested CLS as a cause of death in Patient 1. Gram-negative septicaemia was felt to be excluded as blood cultures were repeatedly negative and prophylactic antibiotics were used. There was no serological nor post-mortem evidence of viral infection. Methotrexate is reported to cause a fatal pneumonitis which may resemble CLS, typically following large cumulative doses,[3] but histopathological examination at post-mortem was not consistent with this diagnosis. Patient 2 developed breathlessness with radiological evidence of pulmonary oedema suggestive of CLS, coincident with recurring episodes of generalized cutaneous pustulation. Pulmonary fibrosis as a sequel of CLS is well documented[4] and it seems likely that recurring episodes of acute pulmonary inflammation accelerated the underlying fibrotic condition that led to respiratory failure in this patient.

Generalized pustular psoriasis is an acute inflammatory dermatosis typically associated with systemic features such as fever, arthralgia, leucocytosis, diarrhoea and altered liver function.[5] We propose that cytokines synthesized in the skin in cutaneous disease might mediate systemic inflammation by interaction with receptors on distant target tissues, for example the bone marrow, liver, synovium and intestinal epithelium. In particular, the action of cytokines on pulmonary vasculature in our patients might account for the development of CLS coincident with generalized pustular psoriasis.

In support of this hypothesis, it is now established that activated keratinocytes elaborate a range of cytokines *in vitro* including interleukin (IL)-1, IL-6, IL-8 and TNF-α and altered expression of these peptides occurs in cutaneous inflammatory disease, including psoriasis.[6,7] Furthermore, elevated levels of IL-1, IL-6 and TNF-α have been demonstrated in the serum of patients with psoriasis and following UVB exposure,[8,9] suggesting that cytokines synthesized by keratinocytes are released into the circulation during cutaneous inflammation. Because IL-6 and TNF-α are known to induce fever, leucocytosis and acute-phase proteins[10] it seems likely that extracutaneous manifestations of pustular psoriasis result from the systemic action of these peptides.

Cytokines are important mediators of pulmonary inflammation[11] and recent studies suggest that increased pulmonary vascular permeability, pathognomonic of CLS, is mediated by TNF-α and IL-1.[12] However, CLS also characteristically complicates intravenous IL-2 administration[13] during treatment of solid tumours, suggesting that a number of interacting cytokines may be involved in the pathogenesis of this condition.

Cytokines are now well established as hormone-like mediators of both cutaneous and pulmonary inflammation and it seems probable that peptides synthesized in the skin during cutaneous inflammation could also act as mediators of systemic inflammation. Previous reports of pulmonary inflammation coincident with generalized pustular psoriasis[14–16] were diagnosed as sterile pneumonitis or unexplained alveolar shadowing but these conditions may represent a similar pathological process. In addition pneumonia and cardiac failure, allegedly the commonest causes of morbidity and mortality in patients with erythrodermic dermatoses,[1] clinically and radiologically resemble CLS and might obscure the real incidence of this inflammatory process as a complication of severe extensive cutaneous inflammation.

References

1 Marks J. Erythroderma and its management. *Clin Exp Dermatol* 1982, 7: 415–22.

2 Teelucksingh S, Padfield PC, Edwards CR. Systemic capillary leak syndrome. *Q J Med* 1990, 75: 515–24

3 Zitnik RJ, Cooper JA. Pulmonary disease due to anti-rheumatic agents. *Clin Chest Med* 1990; 11: 139–50.

4 Pratt PC, Vollmer RT, Shelbourne JD, Crapo JD Pulmonary morphology in the multi-hospital collaborative extracorporeal membrane oxygenator project *Am J Pathol* 1979; 95: 191–214.

5 Lyons JH. Generalised pustular psoriasis *Int J Dermatol* 1987; 7: 409–18.

6 Barker JNWN, Mitra RS, Griffiths CEM *et al.* Keratinocytes as initiators of inflammation *Lancet* 1991; 337: 211–14.

7 Nickoloff BJ, Karabin GD, Barker JNWN *et al* Cellular localisation of interleukin-8 and its inducer, tumour necrosis factor-alpha in psoriasis *Am J Pathol* 1991; 138: 129–37.

8 Kock A, Schwarz T, Kirnbauer R *et al* Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light *J Exp Med* 1990, 172: 1609–14

9 Schwarz T, Luger TA Effect of UV irradiation on epidermal cell cytokine production. *J Photochem Photobiol* 1989; 4: 1–13.

10 Wolvekamp MC, Marquet RL. Interleukin-6: historical background, genetics and biological significance. *Immunol Lett* 1990, 24: 1–9

11 Kelley J Cytokines of the lung. *Am Rev Respir Dis* 1990, 141: 765–87.

12 Tracey KJ, Lowry SF, Cerami A. Cachetin/TNFα in septic shock and adult respiratory distress syndrome. *Am Rev Resp Dis* 1988, 138: 1377–9.

13 Lee R, Lotze M, Skibber J *et al.* Cardiorespiratory effects of immunotherapy with interleukin-2. *J Clin Oncol* 1989; 7: 7–20

14 Landry M, Muller S. Generalised pustular psoriasis: observations on the course of the disease in a familial occurrence *Arch Dermatol* 1972; 105: 711–16.

15 Reed WB Pustular psoriasis of Zumbusch, generalised *Arch Dermatol* 1973, 107: 621.

16 Gordon M, Pearlstein HH, Burgoon CF Pustular psoriasis (Zumbusch). *Dermatologica* 1969; 138: 65–74

British Journal of Dermatology (1991) **125**, 475–476.

Eruptive vellus hair cysts with sebaceous glands

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Accepted for publication 28 May 1991

Summary A patient is described who had eruptive vellus hair cysts with and without sebaceous glands in the cyst walls. An association is suggested between eruptive vellus hair cysts and the condition of steatocystoma multiplex.

Since the original description of eruptive vellus hair cysts (EVHC) in 1977,[1] several cases have been reported which were either sporadic[2–4] or with a familial pattern of inheritance.[5–7] Although EVHC may clinically resemble steatocystoma multiplex, the condition differs histologically in that sebaceous glands are absent. Recently, a case of EVHC with sebaceous glands in the cyst wall has been reported[8] and a mixed form of both EVHC and steatocystoma multiplex has been suggested. We report a further patient who had EVHC with evidence on the histology of sebaceous glands in the cyst wall.

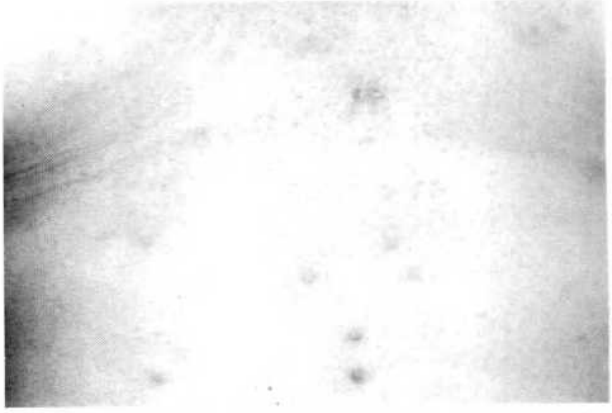

Figure 1. Multiple pigmented pin-sized smooth papules on anterior chest.

Case report

A 21-year-old Japanese woman presented with persistent asymptomatic papular lesions on the thighs, groins and on the front of the chest that had been present for at least 6 years. There was no family history of similar lesions. On examination there were multiple blue–black pin-sized smooth papules on the posterior aspect of both thighs, in the groins and on the front of the chest (Fig. 1).

A skin biopsy was taken from an affected area on the right thigh and showed a cyst in the mid-dermis that was lined by squamous epithelium with a normal overlying epidermis. The cyst was filled with laminated keratinous material and there were numerous vellus hair shafts present (Figs 2 and 3). There was no connection between the cysts and the epidermis and on serial sectioning there appeared to be no attachment to a sebaceous gland. A biopsy from the anterior chest showed a lobular cystic lesion in the mid-dermis with a normal overlying epidermis. On one side of the cyst there was amorphous keratinous material, and on both sides numerous vellus hair shafts were lined by two or three layers of epithe-

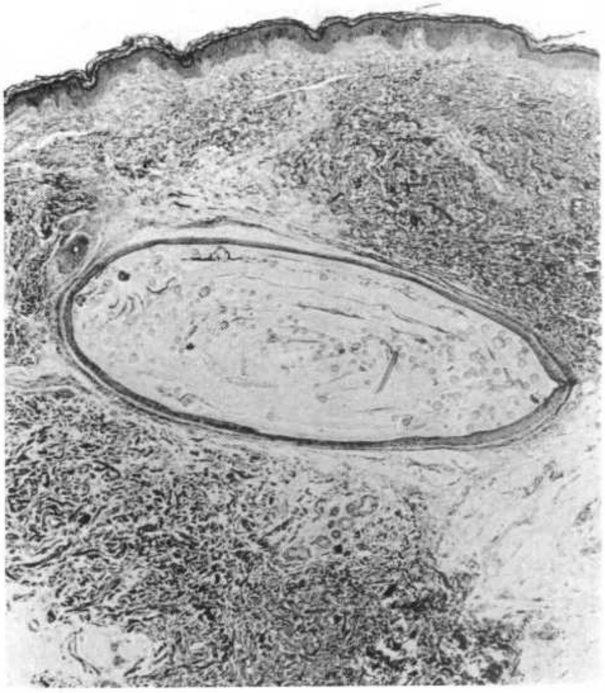

Figure 2. Thin-walled cyst in the mid-dermis containing keratinous material and numerous vellus hairs (haematoxylin and eosin, × 40).

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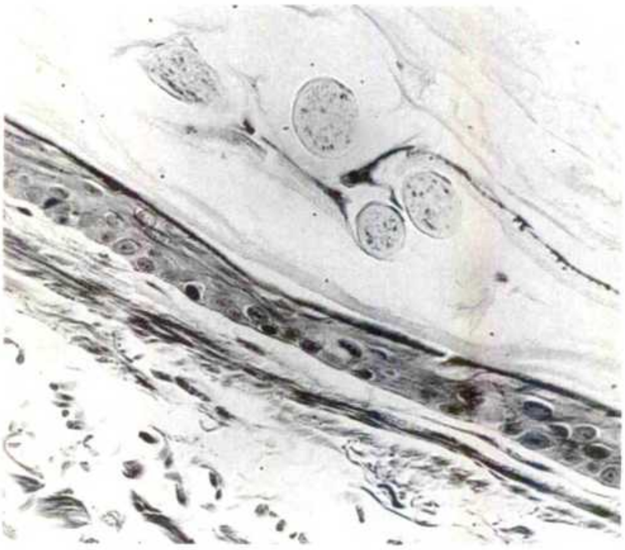

Figure 3. Higher magnification showing cyst lined with squamous epithelium and containing vellus hairs (haematoxylin and eosin, × 150).

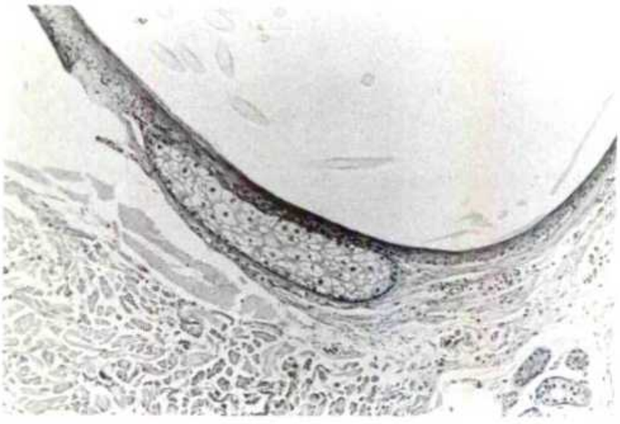

Figure 4. Portion of thin-walled cyst with sebaceous gland attached (haematoxylin and eosin, × 100).

lium. Serial sections showed sebaceous glands in the wall lining the cyst (Fig. 4).

Discussion

Eruptive vellus hair cysts and steatocystoma multiplex are both rare inherited conditions that are characterized by the formation of multiple cutaneous cysts. The age of onset in both disorders is the same and there is a similar distribution of the sites of the lesions. Differentiation between the two disorders can only be made on the histology. In steatocystoma multiplex there is an empty cyst with a thin wall of stratified squamous epithelium and the main differentiating feature from EVHC is the presence of sebaceous glands within the cyst wall with a prominent acellular hyalinized cuticle with undulant configuration.[9] Occasionally the cavity contains vellus hairs[10] and follicular structures.[11] In EVHC the cyst wall is composed of an epithelium without any connection to sebaceous glands and the cavity contains multiple vellus hairs. However, sebaceous glands have been observed near the cysts in EVHC,[12] and a case was reported in which the epithelium lining the cyst had sebaceous gland cells.[5] Two similar cases have been reported and it is suggested that EVHC is closely related to steatocystoma multiplex.[13] Recently, a case has been reported of a patient with both EVHC and steatocystoma multiplex.[14] Our case is also unusual with the presence of sebaceous glands within the cyst wall of the lesions of EVHC.

References

1 Esterly NB, Fretzin DF, Pinkus H. Eruptive vellus hair cysts. *Arch Dermatol* 1977; **113:** 500–3.

2 Bovenmyer DA. Eruptive vellus hair cysts. *Arch Dermatol* 1979; 115: 338–9.

3 Lee S, Kim JG. Eruptive vellus hair cyst: clinical and histological findings. *Arch Dermatol* 1979; **115:** 744–6.

4 Burns DA, Calnan CD. Eruptive vellus hair cysts. *Clin Exp Dermatol* 1981; **6:** 209–13.

5 Stiefler RE, Bergfeld WF. Eruptive vellus hair cysts—an inherited disorder. *J Am Acad Dermatol* 1980; **3:** 425–9.

6 Piepkorn NW, Clark L, Lombardi DL. A kindred with congenital vellus hair cysts. *J Am Acad Dermatol* 1981; **5:** 661–5.

7 Hornstein E, Wilcox C, Oberlies D *et al.* Eruptive vellus hair cyst. *Int J Dermatol* 1986; **25:** 395–6.

8 Urbina Gonzalez F, Cristobal-Gil MC, Aguilar Martinez A *et al.* Eruptive vellus hair cyst with singular histologic finding. *Arch Dermatol* 1987; **123:** 299–300.

9 Brownstein MH. Steatocystoma simplex. A solitary steatocystoma. *Arch Dermatol* 1982; **118:** 409.

10 Egbert BM, Price WM, Segal RJ. Steatocystoma multiplex: report of a case and a review. *Arch Dermatol* 1979; **115:** 334–5.

11 Brownstein MH, Helwig EB. Subcutaneous dermoid cysts. *Arch Dermatol* 1973; **107:** 237–9.

12 Lee S, Kim SG, Kang JS. Eruptive vellus hair cysts. *Arch Dermatol* 1984; **120:** 1191–5.

13 Sanchez-Yus E, Aguilar-Martinez A, Cristobal-Gil MC *et al.* Eruptive vellus hair cysts and steatocystoma multiplex: two related conditions? *J Cutan Pathol* 1988; **15:** 40–2.

14 Jerasutus S, Suvanprakorn P, Sombatworapat W. Eruptive vellus hair cyst and steatocystoma multiplex. *J Am Acad Dermatol* 1989; **20:** 292–3.

British Journal of Dermatology (1991) 125, 477–495.

SOCIETY PROCEEDINGS

British Society for Investigative Dermatology Annual Meeting, Leicester, 26–27 September 1991

Papers

HUMAN PAPILLOMAVIRUS (HPV) AND CUTANEOUS MALIGNANCY IN RENAL TRANSPLANT RECIPIENTS: CLINICOPATHOLOGICAL CORRELATIONS AND CYTOKERATIN EXPRESSION.

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The long term immunosuppression required to prevent renal allograft rejection leads to numerous cutaneous problems. Of particular concern is the very high rate of HPV infection, as there is increasing evidence that this is aetiologically linked to the increased incidence of squamous cell carcinoma (SCC) in these patients.

We have examined 193 renal allograft recipients with a mean time since transplantation of 4.4 years. Overall 48% have viral warts and 22% have had one or more cutaneous malignancy. The prevalence in those patients with type I or type II skin, transplanted 5 or more years previously is much higher, 93% having viral warts and 59% one or more epidermal malignancy, 29% of these with multiple SCC. Both the warts and the SCC are principally on sun exposed skin. We have observed SCC arising from specific warts and from clinically dysplastic skin sheeted with warts. Clinicopathological studies in these patients demonstrate a spectrum of epithelial transformation from benign verruca vulgaris through increasing degrees of squamous atypia to full thickness dysplasia and invasive SCC, with some lesions showing dysplastic change within a benign wart.

Using a panel of monospecific monoclonal antibodies to epithelial keratins we investigated the influence of HPV on epithelial cytokeratin expression and its possible significance in malignant transformation. The simple epithelial keratins 7, 8 and 18, previously shown to be markers of invasive malignancy, were not expressed in these HPV associated malignancies. However, in both dyplastic and invasive lesions patchy suprabasal expression of keratin 17,sometimes seen in benign warts, was prominent and in places panepidermal. Our studies suggest that this expression of keratin 17 may be a novel marker of dysplastic epithelial transformation.

INTRACELLULAR LOCALISATION BY IMMUNOELECTRON MICROSCOPY OF HUMAN PAPILLOMAVIRUS TYPE 16 PROTEINS USING AN *IN VIVO* CULTURE SYSTEM.

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The human papillomavirus (HPV) causes warts, but is also associated with the development of squamous dysplasia and carcinoma in the skin, lower genital tract and upper respiratory tract mucosa. Interactions between the cell and the virus affect epithelial proliferation as well as virus gene expression and replication. In tissue culture, the virus is unable to produce progeny, leading to the assumption that the completion of the virus life cycle is dependent on the state of differentiation and keratinisation of the host cell.

Using an *in vivo* culture system in which human keratinocytes are grafted onto the flank of athymic mice, we have shown that an established line of cervical keratinocytes, W12 cells, containing 50-100 copies per cell of episomal HPV type 16 DNA, are permissive for the production of new virus particles (Sterling J, Stanley M, Gatward G *et al*. Production of human papillomavirus type 16 virions in a keratinocyte cell line. *J Virol*. 1990, 64, 6304-6307) Immunoelectron microscopy of a seven week graft using a mouse monoclonal antibody against the HPV 16 L1 (major capsid) protein, confirms that these particles are HPV type 16. This capsid protein is found only in condensed nuclei of the upper granular and cornified layers. The viral E4 protein, which is postulated to be important in the early stages of capsid production, is localised to the cytoplasm in certain cells of the granular layer.

This system appears to parallel many features of HPV-infected epithelium and should therefore provide a useful experimental approach to the study of the complete virus life cycle and its effects on the keratinocyte.

SPONTANEOUSLY IMMORTALISED KERATINOCYTE CELL LINES FROM A RENAL TRANSPLANT RECIPIENT: A MODEL FOR MALIGNANT TRANSFORMATION OF KERATINOCYTES.

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The association of oncogenic types of human papillomavirus (HPV) with cervical carcinoma is well established Specific HPV types appear to play a similar role in malignant transformation of skin, especially of light-exposed skin in immunosuppressed allograft recipients. The role of wart virus in cellular transformation is poorly understood; not least because this strictly epitheliotropic virus cannot be cultured in vitro. The degree of epithelial differentiation required to switch on viral particle production is not achieved with standard keratinocyte culture techniques. We describe immortalised cell lines showing abnormal keratinocyte differentiation on complex substrates and providing an *in vitro* model for studying this malignant transformation.

Tissue culture of dysplastic forehead skin from a renal allograft recipient with multiple squamous cell carcinomas arising from warts or wart-ridden skin led to the establishment of 7 spontaneously immortalised keratinocyte lines. Characterisation of these lines finds them dependent on a fibroblast feeder layer and standard keratinocyte growth media plus foetal calf serum. At early passage number (<15) none of these lines have been tumourigenic in immunocompromised mice while karyotype analysis found polyploidy in a minority It is likely that these cells will become more "transformed" with continued growth in culture. Karyotype, tumourigenesis and growth factor requirements will be repeated at a later passage.

Growth of these keratinocyte lines on complex substrates produces a multilayered epithelium with grossly abnormal keratinocyte differentiation morphologically. Markers of differentiation have been assessed using a panel of well characterised monospecific monoclonal antibodies to epithelial keratins. Differentiation-specific cutaneous keratins 1 and 10 were lost while keratin 17 was strongly expressed in almost all cases. These cell lines offer a valuable *in vitro* system for studying the mechanisms underlying and factors influencing viral transformation of keratinocytes

IN VITRO TRANSFORMATION OF ORAL KERATINOCYTES BY HUMAN PAPILLOMAVIRUS.

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Transformation of genital keratinocytes by human papillomavirus (HPV) is well documented both *in vivo* and *in vitro*. In this region, HPV16 and 18 have been designated as "transforming" and are associated with the majority of cervical carcinomas, while HPV6 and 11 are found in benign warts. Genital keratinocytes can be immortalised *in vitro* by transfection with HPV DNA but this ability is similarly restricted to the "transforming" HPV types. HPV is known to infect oral and laryngeal keratinocytes *in vivo* and has been documented in some oral carcinomas; yet an oncogenic role for HPV16/18 in oral lesions is not established and *in vitro* immortalisation has not previously been attempted. We therefore compared the transforming potential of HPV16 and HPV11 in cultured oral keratinocytes

Using whole genomic HPV DNA in the pJ4 omega plasmid construct and lipofection transfection we can routinely immortalise oral keratinocytes with HPV16 but not, despite identical conditions, with HPV11. HPV16 DNA and E7 protein were detected in all immortalised lines To date, none of these lines have been tumourigenic in immunocompromised mice. All the HPV16 cell lines appeared to undergo profound "crisis" after 3 months in tissue culture but subsequently have grown well Markers of differentiation have been examined using a panel of well characterised monospecific monoclonal antibodies to epithelial keratins In monolayer cultures there was no significant alteration in keratin expression when compared to normal oral keratinocytes These experiments demonstrate that the immortalising activity of HPV16 is not restricted to the genital region and that immortalisation with HPV11 is not, under these circumstances, enhanced by regional differences in keratinocytes

CHARACTERIZATION OF A HUMAN KERATIN GENE (HK?) LINKED TO HK1.

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Keratin gene expression is complex and tightly regulated in human epidermis. While almost thirty keratin proteins have now been described in total, only 10 are expressed in the epidermis. Many of the genes that encode these keratins have now been characterized at the molecular level, and have been localised to two multi-gene clusters, one on chromosome 12 (mainly type II genes) and the other on chromosome 17 (type I genes). We have now characterized another type II keratin gene (HK?) that is similar but not identical to HK1 and lies 10 Kb upstream of the HK1 transcription start site, both being part of the chromosome 12 gene cluster.

The HK? gene was isolated on the same cosmid fragment (14-1a) as the HK1 gene and its regulatory region. The gene was located on the 5'end of the cosmid DNA and lay on a single Bam HI fragment (6F8, 14 Kb). The Eco RI fragments that covered this region (4C2, 9.4 Kb; 1E6, 0.6 Kb; 1E11, 5.1 Kb; 4D11, 4.9 Kb) were subcloned, restriction mapped and sequenced by the dideoxy method using oligo primed dsDNA. In addition, parts of 1E11 were further subcloned to allow sequencing.

The coding region of the gene was smaller than HK1 but due to the longer introns, the gene was larger overall. HK? spanned 9680 bp from the ATG (Exon 1) to the polyadenylation signal. The exon structure coding for the helical portions of the protein was almost identical to HK1, but exons 1 and 9 which encode the N- and C-termini, were smaller. The amino acid sequence was 65% homologous to HK1 and while the termini were glycine-rich, similar to HK1 and HK10, the region prior to the stop codon was unique.

Thus, the sequence data that we have obtained for HK?, a type II human keratin gene that is linked to HK1 (10 Kb upstream), shows that this gene is similar but not identical to HK1. While the sequence data does not indicate that this is a pseudogene, it is not yet known whether HK? is expressed in the epidermis. However, probes have now been contructed to examine this question.

POINT MUTATIONS OF THE N RAS ONCOGENE IN HUMAN MALIGNANT MELANOMA AND POTENTIAL PRECURSOR LESIONS.

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The aim of this study is to identify the frequency of point mutations of the N ras oncogene in human malignant melanoma and possible precursor lesions, and also to establish whether or not such point mutations are found preferentially in lesions arising on habitually light-exposed skin.

Tissue sections have been cut from blocks of primary malignant melanoma (28 cases: 19 F, 9 M: 22 or 78% on exposed skin), secondary melanoma (4), dysplastic naevi with cytological atypia (18), and small congenital naevi (13). After extracting DNA from this material, it has been amplified using the polymerase chain reaction, in the presence of specific amplimers for codons 12/13 and 61 of the N ras oncogene. Amplified product has then been probed with ^{32}P-labelled oligonucleotides, using the dot blot technique. Material showing specific point mutations is then sequenced directly to confirm the presence of the identified point mutation.

Point mutations in codons 12/13 or 61 of N ras have to date been identified in 2/4 secondary melanomas (50%), 4/28 primary melanomas (14%), 3/13 small congenital naevi (26%), and 0/18 dysplastic naevi. Point mutations in primary melanomas were all in females and were on exposed sites in 3/4 cases (75%).

These results indicate that point mutations in codons 12/13 and 61 of N ras are found in 1/7 of primary melanomas, and are not preferentially found in lesions arising on sun-exposed skin. This is the first study to detect point mutations in small congenital naevi, and may be a further indication of the premalignant potential of some of these lesions. They also indicate that N ras mutations are neither necessary nor sufficient for malignant transformation of melanocytes.

POLYCYCLIC AROMATIC HYDROCARBON INDUCED DNA-DAMAGE IN HUMAN SKIN AND SKIN EQUIVALENTS.

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The majority of chemical carcinogens exert their mutagenic effects by forming reactive electrophilic species which bind to cellular DNA. We have utilised the HPLC-^{32}P post-labelling method of DNA-adduct detection to investigate polycyclic aromatic hydrocarbon induced DNA damage in human skin explants and skin equivalents.

The formation of DNA-adducts was measured in human skin and skin equivalents by incubating the tissue with 2μM benzo[α]pyrene or 1,6-dinitropyrene for 24 or 48 hours at 37°C in an atmosphere of 5% CO_2 in air. Cellular DNA was extracted, purified and briefly treated with nuclease P1. The DNA-adducts were 5'-phosphorylated with polynucleotide kinase and [γ-^{32}P]ATP and the radiolabelled DNA-adducts analysed by reverse-phase HPLC. The chromatographic profiles of the DNA-adducts extracted from human skin and skin equivalents treated with either B[α]P or 1,6-DNP were very similar. Two DNA-adduct species were detected in 1,6-DNP treated human skin explants and skin equivalents. These two DNA-adducts co-eluted with standard 1,6-DNP induced DNA-adducts synthesised by reacting calf thymus DNA with 1,6-DNP in the presence of cytochrome oxidase and acetyl Co-enzyme A. B[α]P induced DNA-adducts were also detected in human skin and skin equivalents with the most abundant being (+)-7R-trans-(anti)-BPDE-dGuo. The amount of each B[α]P and 1,6-DNP adduct extracted from both tissues increased with the time of exposure. However, the human skin explants showed some variation in the amount of each adduct formed.

The data show that the ^{32}P post-labelling method is a versatile and sensitive method of DNA-adduct detection in human skin. The results also suggest that the skin equivalent provides one with a valid in vitro model for the estimation of carcinogenic risk as it retains the enzyme systems required for the activation of polycyclic aromatic hydrocarbons.

EPITHELIAL PHENOTYPE IS AN INTRINSIC PROPERTY OF KERATINOCYTES BUT IS MODULATED BY ENVIRONMENTAL FACTORS.

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We investigated whether the variations in phenotype of epithelia from different sites were intrinsic or induced, by comparing the morphological and functional characteristics of epidermal and oral keratinocytes in the absence of connective tissue. Keratinocytes from human buccal mucosa and skin were seeded on to type I collagen membranes (Cellagen - ICN Flow, USA) and grown as submerged (sub) or interface (i/f) cultures in supplemented DMEM/Ham F12. After 10 days, permeability coefficients (Kp; x 10^{-7} cm/min) of the confluent stratified keratinocyte sheets to water was determined. Control permeability values were obtained using biopsies of intact human buccal mucosa and skin. The permeability coefficients of cultured (sub = 505 ± 48, i/f = 455 ± 52) and intact (583 ± 29) buccal mucosa were similar. However, cultured skin showed significantly higher Kp values (sub = 416 ± 55, i/f = 282 ± 24) than normal skin (52 ± 9) (p 0.05). Electron microscopy showed stratification of epidermal and buccal mucosal keratinocytes in vitro. Membrane coating granules (MCGs) characteristic of non-keratinising epithelia were seen with cultures of both cell types. These were similar in number and distribution in both intact and cultured buccal mucosa. Epidermal keratinocytes formed only small numbers of atypical MCGs.

These results indicate that keratinocytes from different sites exhibit intrinsic phenotypic differences, but that these may require the appropriate environmental signal for their full expression.

Miss L. Balasubramaniam is in receipt of a Unilever Research Scholarship

QUANTIFICATION AND LOCALISATION OF LIPOCORTIN-1 IN NORMAL HUMAN SKIN.

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Lipocortin-1 (Lc1) is a member of the annexin family of calcium dependent phospholipid binding proteins. Lc-1 has been reported to inhibit PLA_2 activity *in vitro*, although the mechanism of this action and its physiological relevance is unclear. The recombinant human protein has profound antiinflammatory activity in animal models and antagonises IL-1 induced fever in mice. Lc-1 is produced by human monocytes *in vivo* in response to intravenous corticosteroid and may mediate some of the antiinflammatory effects of these drugs (1), however the mechanisms of its antiinflammatory actions and its role in inflammatory diseases of the skin have yet to be investigated.

In this study we report the distribution of Lc-1 in normal skin and have quantified the amount of the protein in epidermis and dermis using a specific ELISA.

Samples of normal abdominal skin were taken at post-mortem and snap frozen. Different samples from each individual were either sectioned by cryostat or prepared for Lc-1 extraction. This procedure involved removing excess fat and separating the epidermis and dermis by soaking in 1M NaCl containing 2mM $CaCl_2$. The wet weight of each sample was estimated before microhomogenisation in phosphate-buffered saline containing 10 mM EDTA. Results were expressed as total amount of Lc-1 per mg of tissue or as a % of total protein. The following table shows that Lc-1 is present in both epidermis and dermis.

	ng Lc-1	/ mg tissue		% of tot prot.	x 10^{-3}	
	n	Median	Range	n	Median	Range
Epidermis	15	1.6	0.6 - 8.8	15	3.2	0.9 - 18.2
Dermis	12	0.3	0.1 - 0.9	15	2.0	1.5 - 8.6

Lc-1 concentration did not alter with age in normal subjects; median age 69 years (range 25 - 80); neither was there any sex difference.

An alkaline phosphatase / anti-alkaline phosphatase amplified immunohistological staining method was used on the frozen sections of post mortem normal skin. This confirmed the quantitative studies by revealing strongly positive Lc-1 staining within the epidermal basal layer. Much less staining was observed within the dermis, although positive staining could be seen associated with fibroblasts and related to sweat glands.

(1) Goulding NJ., Godolphin JL., Sharland PR., et al Anti-inflammatory lipocortin-1 is produced by peripheral blood leucocytes in response to intravenous hydrocortisone Lancet 1990. 335,1416-1419

INHIBITION OF EGF-STIMULATED PHOSPHOLIPASE A2 ACTIVITY BY THE TYRPHOSTIN AG213 IN LESION-FREE PSORIATIC EPIDERMIS.

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A biochemical link between EGF-receptor kinase activity and phosphorylation of intracellular proteins is now generally accepted. Phosphorylation of lipocortin relieves its inhibition of phospholipase A2 (PLA2) and hence increases the production of free arachidonic acid and inflammatory eicosanoids. A family of tyrosine analogues, termed tyrphostins, has been shown to specifically inhibit EGF-receptor kinase (reviewed in Posner I, Gazit A, Gilon C and Levitski A. FEBS Letters 1989, 257, 287-291). Here we report the effect of the tyrphostin AG213 on PLA2 activity in lesion-free psoriatic epidermis.

Skin slices were obtained using a Davies dermatome and ethyl chloride local anaesthesia from the upper arm in four volunteers with chronic plaque psoriasis. Skin specimens were obtained at least 5 cm from the nearest lesion. Skin slices were maintained at 37°C for 2 h. in Dulbecco's modified Eagles'/F12 Ham medium containing EGF(10 nM); EGF(10 nM) plus AG213(100 μM); AG213(100 μM) or plain medium. Extracts of epidermis was assayed for PLA2 activity and protein as previously described.

For samples maintained in the presence of EGF, the mean PLA2 activity ± S.E.M. was 97.7 ± 6.6 nmol/mg protein/hour (n = 4). The inclusion of AG213 reduced this activity to 81.5 ± 4.1, which difference is statistically significant ($P < 0.05$, paired t test). The remaining two treatments yielded PLA2 activities of 82 4 ± 4.7 (AG213) and 83.2 ± 3 9 (plain medium).

These results demonstrate that the tyrphostin AG213 inhibits EGF-stimulated PLA2 activity in lesion-free psoriatic epidermis. This tyrphostin is thus a candidate drug for the treatment of psoriasis and a phase I clinical trial is reported to be in progress (Corda D, Luini A and Garattini S. Trends in Pharmacological Sciences 1990, 11, 471-473)

The financial support of the Psoriasis Association is gratefully acknowledged.

EPIDERMAL GROWTH FACTOR ACTIVATES PROTEIN KINASE C IN HUMAN DERMAL FIBROBLASTS BUT NOT IN HUMAN KERATINOCYTES.

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Epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) are potent mitogens for human dermal fibroblasts (DF's) and human keratinocytes (KC's). TGF-α is overexpressed in psoriatic epidermis and may contribute to the epidermal hyperproliferation in this disease. The actions of EGF and TGF-α are mediated principally through the EGF receptor. In transformed squamous cell lines that overexpress the EGF receptor, (e.g. A431 cells), EGF activates the phospholipase C/protein kinase C (PLC/PKC) signal transduction pathway as demonstrated by inositol trisphosphate (IP_3) formation and PKC activation. We were therefore interested to investigate whether EGF/TGF-α activated the PLC/PKC pathway in normal human DF's and KC's

The MARCKS protein has recently been characterized as a prominent cellular substrate for PKC. TPA (50nM), a known activator of PKC, induced a 10 fold increase in MARCKS protein phosphorylation in human DF's (n=5) and a 2 fold increase in KC's (n=6). In human DF's a 5 fold increase in MARCKS protein phosphorylation was observed in response to EGF (50nM) and TGF-α (50nM), (n=5) reaching a peak at 5 mins. EGF induced MARCKS phosphorylation was inhibited by prior treatment with (i) bryostatin to down-regulate PKC and (ii) the PKC inhibitor H7, suggesting a PKC dependent pathway. In contrast, neither EGF or TGF-α significantly induced MARCKS phosphorylation in human KC's (n=6).

PLC hydrolyses phosphatidylinositol-4,5-bisphosphate to yield IP_3 and diacylglycerol (DAG), the physiological activator of PKC. EGF/TGF-α did not significantly induce IP_3 formation in human KC's (n=7) or DF's (n=6), as measured by a 1,4,5-IP_3 binding assay and [^{3}H]IP_3 release from [^{3}H]myo-inositol labelled cells. We have previously shown elevation of DAG due to hydrolysis of phosphatidylcholine in response to EGF in human DF's (Fisher GJ et al. J Cell Physiol, 1991;146:309-317). In human KC's, however, no elevation of DAG or evidence of phosphatidylcholine hydrolysis was observed in response to TGF-α or EGF, (n=2).

Thus EGF and TGF-α significantly induced PKC activation in human dermal fibroblasts, and thus occurs primarily through phosphatidylcholine hydrolysis. In human KC, however, neither EGF or TGF-α significantly activated PKC or induced hydrolysis of phosphatidylinositol or phosphatidylcholine. These data demonstrate that signal transduction pathways in normal cells differ from those in transformed cells and furthermore that DF's and KC's exhibit distinct signalling pathways in response to EGF.

CHANGES IN INTRACELLULAR FREE CALCIUM OF HUMAN KERATINOCYTES DURING GROWTH INHIBITION BY VITAMIN D_3.

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Human keratinocytes differentiate following an increase in the extracellular calcium concentration ($[Ca^{++}]_o$) and this effect is preceded by an early rise in intracellular free calcium ($[Ca^{++}]_i$) (Sharpe et al, FEBS Letters 1989; **254**;25-8). Vitamin D_3 (1α,25(OH)$_2$ cholecalciferol) also induces differentiation and we have investigated the changes in growth and $[Ca^{++}]_i$.

Human keratinocytes were grown in the serum free medium MCDB153 ($[Ca^{++}]_o$ 70μM) and growth inhibition measured by ^{3}H-thymidine incorporation. Labelling (corrected for DNA content of cultures) was reduced to 29% of control cultures after 4 days D_3 10^{-6}M (n=4). These findings were confirmed by cell counting studies; cells counts were reduced to 68±4% and 52±3% (mean ± SEM, n=6) of control cultures after 5 days D_3 10^{-8}M and 10^{-7}M, respectively. The results demonstrated a dose-time dependent inhibition of growth and induction of morphological changes. With 10^{-6}M D_3 morphological changes were seen at 3 days, at 5 days with 10^{-7}M D_3 and 6-8 days at lower concentrations.

The $[Ca^{++}]_i$ within single keratinocytes was measured using the Ca^{++} sensitive fluorescent dye FURA-2 with a photon counting system and microspectro-fluorometric techniques. The $[Ca^{++}]_i$ in control cells was 104±3 nM (n=40). No immediate rise in $[Ca^{++}]_i$ was demonstrated within the first 10 mins of addition of D_3 up to 10^{-6}M, but occurred after an increase in $[Ca^{++}]_o$ to 2mM. There was however, a rise to 141±5 nM (n=30) at 8 hrs, with a further increase to 173±9 nM (n=40) at 24 hrs. The increase was sustained at 48 hrs and similar to the sustained rise following an increase in $[Ca^{++}]_o$ to 2mM (166±5 nM at 24 hrs, n=29).

The results confirm that treatment of keratinocytes with D_3 induces a sustained rise in $[Ca^{++}]_i$ preceding the morphological changes and growth reduction. The increase after 6-8 hrs is however different from the immediate rise, within minutes, previously demonstrated with an increase in $[Ca^{++}]_o$. This is consistent with D_3 acting by alteration of gene expression.

INFLUENCE OF TUMOUR NECROSIS FACTOR α ON DENDRITIC CELL ACCUMULATION IN LYMPH NODES.

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Freshly isolated Langerhans cells (LC) are relatively inefficient antigen presenting cells. It has been found, however, that during culture this property develops and is associated with a significant elevation in expression of membrane major histocompatibility (MHC) class II (Ia) antigen. This maturation is mediated, at least in part, by granulocyte/macrophage colony-stimulating factor (GM-CSF) and possibly interleukin-1 (IL-1) (Heufler C, Koch F and Schuler G. J. Exp. Med. 1988; 167 : 700-705).

Following topical exposure to skin sensitizing chemicals LC are induced to migrate to the draining lymph nodes. We have recently found that this migration is accompanied by a similar increase in Ia expression. As GM-CSF and IL-1 are products of keratinocytes the implication is that during migration LC undergo a maturation similar to that observed in vitro.

In the present study we have examined whether in addition to possibly influencing the functional and phenotypic characteristics of LC, epidermal cytokines also provide a stimulus for LC to migrate from the skin. The effects of tumour necrosis factor α (TNFα) on the accumulation of dendritic cells (DC) in draining lymph nodes has been investigated.

Groups of mice (n=10) received recombinant murine TNFα (50ng/2000u) in the dermis of each ear. At various times thereafter draining lymph nodes were excised and the number of DC per node measured. A 2.8 to 3.6 - fold increase in the frequency of draining node DC was observed as early as 4 hours following treatment. The response was species specific in as much as exposure under the same conditions to 50ng of recombinant human TNFα (of approximately the same specific activity in vitro) failed to induce similar changes. Equivalent amounts of heterologous protein, or of recombinant murine GM-CSF, also failed to cause significant changes in lymph node DC. The data indicate that TNFα influences the accumulation of DC in draining lymph nodes and may provide one signal for LC migration.

TUMOUR NECROSIS FACTOR-*α* IS PROINFLAMMATORY IN HUMAN SKIN AND MODULES CUTANEOUS ADHESION MOLECULE EXPRESSION.

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Recent evidence suggests that keratinocytes may inducibly produce tumour necrosis factor-*α* (TNF-*α*), and in vitro TNF-*α* is a modulator of many immune and inflammatory processes including endothelial adhesion molecule expression. We have therefore performed an immunohistochemical study of the effects of intradermal recombinant human TNF-*α* (rHuTNF*α*) in normal human skin using monoclonal antibodies to ICAM-1, ELAM-1 and VCAM-1.

Three normal adult male volunteers each received 100U rHuTNF*α* by intradermal injection. An equal volume of normal saline was injected as control and both sites were biopsied at six hours In control skin, no clinical change occured. ICAM-1 was present constitutively on endothelium and occasional interstitial cells, there was minimal endothelial ELAM-1 expression and VCAM-1 was present on perivascular dendritic cells. Following rHuTNFα injection, minimal induration and erythema was present at the injection site after 4-6 hours which resolved by 24 hours There was marked upregulation of ICAM-1 on endothelium and dermal interstitial cells Also, in all cases there was strong ICAM-1 expression by keratinocytes, most strongly in the basal layer. ELAM-1 was upregulated on endothelial cells as was VCAM-1, though to a lesser extent.

This study demonstrates that TNF-α is proinflammatory in normal human skin Modulation of endothelial, dermal cell and keratinocyte adhesion molecules provides a means for inflammatory cell recruitment from the circulation and trafficking within the skin Taken together, these findings suggest that TNF-α may be an important mediator of cutaneous inflammatory responses.

EPIDERMAL LANGERHANS CELLS UNDERGO IN SITU MORPHOLOGIC AND PHENOTYPIC CHANGES IN PSORIASIS.

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Cultured epidermal Langerhans Cells(LC) undergo phenotypic alteration following addition of cytokines in vitro. In particular, loss of CD1a expression, upregulation of MHC Class II antigens and induction of adhesion molecules is observed, associated with increased antigen presenting capacity in vitro. Since cytokines are important mediators of cutaneous inflammation, this immunohistochemical study examines the phenotype of LC in normal and psoriatic skin in vivo

5um cryostat skin sections of normal skin (n=9) and psoriasis (n=9) were stained using a PAP technique for single labelling and a combination of PAP and APAAP methods for double labelling where necessary, for a range of surface determinants

Epidermal dendritic cells in psoriasis demonstrate markedly shortened dendritic processes and loss of CD1a expression (mean no. of cells 6.4+/-0.62 c.f. 11.0+/-0.82 in normal skin)($p<0.05$) Up-regulation of MHC Class II antigens (10.8+/-1.8) c.f. (6 25+/-0 63)($p=0.07$) and induction of β_2-integrins, namely CD11a,CD11c and CD18 was also consistently observed. In contrast to normal skin, CD18+ve LC were observed in psoriasis as demonstrated by double labelling.

This study is consistent with the hypothesis that LC undergo in situ phenotypic changes in psoriasis These changes may reflect functional alteration in LC antigen presenting capacity in vivo.

CYTOKINE-INDUCED IN VITRO LYMPHOCYTE MIGRATION IS NOT DEPENDENT ON CELLULAR ACTIVATION.

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Work in this department has previously demonstrated in vitro peripheral blood lymphocyte (PBL) locomotion in response to an unexpectedly wide range of cytokines and other agonists, including interleukins (ILs) 1, 2, 3, 4, 6, 7 and 8, RANTES, leukotriene B4, 12[R]-HETE, f-met-leu-phe and zymosan activated plasma (ZAP). In order to determine whether activation of PBL during purification enhanced their locomotor responsiveness, in vitro migration in a 48-well microchemotaxis apparatus (Bacon KB et al, Br J Pharmacol 1988; 95: 966-74) was assessed in response to IL-1 alpha, IL-2, IL-8 and ZAP) following two different purification methods (a two-step, 18 h plastic adherence method previously used in demonstrating the responsiveness to the above wide range of agonists and possibly associated with PBL activation, and a rapid, nylon column purification method). Recent work has shown that the locomotor responses of PBL purified by the two methods, to the above four agonists, were dose-related and similar (Pleass R and Camp RDR, J Invest Dermatol, abstract in press). We have now studied the expression of the activation marker, CD25 (IL-2 receptor) on cytospin preparations of PBL purified by the two methods, using monoclonal antibodies. The number of positively labelled PBL are expressed as a percentage of total haematoxylin-counterstained cells (mean ± sem values shown).

PBL purification method	CD25 + (%)	Pan-T marker + (%)
18 h adherence	5 0 ± 0.2 (n=3)	85 ± 2 (n=4)
nylon columns	4 3 ± 0.5 (n=4)	88 ± 1 (n=5)

The expression of CD25 by the PBL preparations was thus similar, the low percentages being comparable to those seen in minimally processed normal peripheral blood. In further experiments, pre-incubation of nylon column purified PBL with anti-CD25 substantially reduced in vitro locomotor responses to IL-2, maximal migration indices (at 10^{-12}M IL-2) being 2 68 ± 0.11 for untreated PBL versus 2 19 ± 0.32 for anti-CD25-treated PBL (mean ± sem, n=4) In contrast, the responses to IL-1 alpha and IL-8 were not affected by anti-CD25 (n=4).

These results support our previous evidence that the in vitro locomotor responsiveness of PBL to a range of agonists is not dependent on artefactual activation of PBL during purification. The responses to IL-2 appear to be through binding to the high affinity IL-2 receptor, which is sufficiently expressed in unactivated, normal PBL to allow migration in response to low concentrations of this cytokine.

CUTANEOUS VASCULAR RESPONSES TO ENDOTHELIN IN NORMAL SKIN AND IN RAYNAUD'S PHENOMENON

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The novel endothelial cell-derived peptide endothelin-1 (ET) has been implicated (*Lancet* 1990; 336· 1144-47) in the pathophysiology of Raynaud's phenomenon (RP) ET elicits intense vasoconstriction after intradermal injection but also causes a secondary vasodilatation peripheral to the pallor (Br J Pharmacol 198; 95. 1005-07). The mechanism of this vasodilatation is not known but may be due to peptidergic nerves A deficiency of calcitonin gene-related peptide (CGRP)-containing nerves has been reported in RP (*Lancet* 1990, 336·1530-33). We have now (1) investigated the role of peptidergic nerves in the vasodilatation caused by ET and (2) compared the pallor and erythema responses of the digital cutaneous microvasculature to ET in RP with those of normal finger skin

(1) Four normal subjects were studied Capsaicin (which depletes nerves of neuropeptides) ointment (2% w/v) was applied for 4 days under occlusion to a 5x10cm area of the anteromedial aspect of one forearm On the 5th day, at an ambient temperature of 20-21° C, intradermal injections of 25µl of 2.5µM ET-1 and normal saline were made within the capsaicin treated area and at corresponding sites on the contralateral forearm The areas of the pallor and erythema responses, measured by planimetry and laser Doppler flowmetry (LDF), 2, 10 and 20 minutes later, showed that the erythema response to ET following capsaicin pretreatment (10 min-area· 140mm² +/- (SEM) 60, LDF. 56% +/- 28) was significantly (Wilcoxon, $p<0\ 05$) diminished compared with normal (area. 1160 +/- 210; LDF. 388 +/- 60) but not the pallor.

(2) Eight women with primary RP and 10 age-matched normal women were studied. At 20° C, baseline cutaneous blood flow was measured over the dorsum of the middle phalanges of one hand using LDF An intradermal injection of 25µl of 2 5µM ET-1 or normal saline vehicle control was randomly made at each site After 2, 10 and 20 minutes, blood flow was again recorded and diameter of visible flare was measured by planimetry The subject was then introduced into a temperature and humidity controlled Environmental Chamber set at 5° C and the experiment repeated on the fingers of the contralateral hand. There were no significant differences between the normal and RP subjects in either their vasoconstrictor responses to ET as measured by pallor size or by LDF (at either 20° C or 5° C), nor in the peripheral erythema measured by LDF However, at 20° C the mean RP erythema responses (20 min-19 9mm +/- (SEM) 2.63) were significantly (Mann-Whitney (MW)· $p<0\ 05$) less than that of the normals (29 8 +/- 3.05). At 5° C the responses in RP (20 min-7 63mm +/- 4 24) were significantly (MW: $p<0.05$) less than in normals (23 4 +/- 4.71) and significantly (Wilcoxon $p<0\ 05$) less than the RP responses at 20° C.

These results (1) support the idea that the erythema due to ET is propagated by peptidergic nerves Because they also show (2) that the size of the erythema response to ET is decreased in RP they lend support to a deficiency of CGRP nerves in RP.

MECHANISMS OF VASODILATATION IN THE CUTANEOUS MICROVASCULATURE.

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Although evidence indicates that neuropeptides such as the vasodilators calcitonin gene-related peptide (CGRP) and substance P (SP) are potentially important regulators of vascular tone, their mechanism of action are unclear. Activation of guanylate cyclase with subsequent increase in intracellular c-GMP levels is one mechanism of vasodilatation which may result either from direct peptide activation or be endothelium dependent and mediated by release of endothelium derived-relaxing factor / nitric oxide (EDRF/NO). The existence of these mechanisms has not been demonstrated in the cutaneous microvasculature, therefore we have sought to determine whether human dermal microvascular endothelial cells (HDMEC) release EDRF and whether release is modulated by either CGRP or SP.

EDRF/NO induces smooth muscle relaxation and blocks agonist induced platelet aggregation by similar mechanisms. In the present experiments we have assessed HDMEC release of EDRF by measuring the ability of HDMEC to inhibit aggregation of washed human platelets induced by the thromboxane analogue U46619. Peptide modulation of platelet aggregation in the absence and presence of HDMEC has also been examined and correlated with investigation of the effect of the peptides on c-GMP levels in HDMEC themselves. Sodium nitroprusside (NP), which inhibits agonist induced platelet aggregation and increases c-GMP levels in target cells was used as a positive control in these experiments.

U46619 induced platelet aggregation was attenuated in the presence of HDMEC (n=3). This effect was concentration dependent and reduced if the platelets were pre-incubated with haemoglobin (Hb)(100μM), an inhibitor of guanylate cyclase. Of the peptides, SP alone induced platelet aggregation at concentrations $>10^{-5}$M. At concentrations $<10^{-5}$M, SP potentiated HDMEC inhibition of platelet aggregation and this effect was blocked by Hb. CGRP (10^{-10}M-10^{-7}M) directly inhibited U46619 induced aggregation in a concentration dependent manner. CGRP was 1000 fold more potent than NP in this respect. The effect of CGRP on platelet aggregation was not potentiated in the presence of HDMEC but was blocked by Hb. In HDMEC incubated with either CGRP or SP, no significant increase in c-GMP was observed. In contrast NP (10^{-5}M-10^{-4}M) increased c-GMP accumulation.

These results indicate that peptide induced vasodilatation in the human cutaneous microvasculature is both endothelium dependent and independent. Furthermore, they indicate that the endothelium dependent mechanism is possibly mediated by release of EDRF/NO from HDMEC.

NEUROPEPTIDE AND NEUROFILAMENT STUDIES IN NODULAR PRURIGO.

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Neurofilament and neuropeptide reactivities were investigated by quantitative and semi-quantitative methods in involved skin in 8 patients with nodular prurigo and in 5 normal control biopsies. Immunohistochemical methods were used employing antibodies to neurofilaments (NF), substance P (SP), calcitonin gene related peptide (CGRP), vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NY). The NF immunoperoxidase reactivity was assessed using a Quantimet 970 image analyser (Cambridge Instruments) and neuropeptide immunofluorescence was evaluated semi-quantitatively. The quantified NF counts for the nodular prurigo patients were higher than the highest control in all except 2 cases. The highest NF counts were seen in nodular prurigo patients with the longest histories of the disease. The means of 3796.5±366.2 SEM (arbitrary units per unit dermal area) and 2454.8±152.8 for the nodular prurigo and control groups respectively were significantly different (student's t test: $p<0.01$). All 8 nodular prurigo biopsies had increased CGRP in comparison to the controls. The CGRP was increased throughout the dermis as single strands or clusters of nerves. SP was increased in 4 out of 8 nodular prurigo biopsies, as single or double strands of nerves. VIP nerve reactivity was concentrated around sweat glands and was increased in 3 out the 8, but NY did not show any difference from the controls. The increased NF reactivity is thought to be a secondary phenomenon but the neuropeptide changes suggest that CGRP and possibly SP and VIP could mediate neurogenic inflammation in nodular prurigo.

STRATUM CORNEUM KINETICS OF TOPICAL TERBINAFINE : FUNGICIDAL CONCENTRATIONS WITH SHORT COURSE THERAPY

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Topical antifungal agents are usually applied until a dermatophyte infection has healed, and then for a further 10 - 14 days. The purpose of this study was to determine the stratum corneum (SC) kinetics of a topical fungicidal drug, terbinafine, during and after therapy, in order to determine whether much shorter courses of therapy may be effective. 20 volunteers (10 males, 10 females, median age 34 years, range 22-62) were randomised to four groups in whom terbinafine 1% cream was applied to the back daily for 1, 3, 5 or 7 days. Up to five sequential skin surface biopsies (SSB) were taken from the upper back at various timepoints during treatment and at 4, 8, 12, 24, 48, 72, 96 and 168 hrs following the last application, and SC terbinafine levels measured using HPLC. Increasing the number of applications from one to seven did not significantly increase the peak SC concentration (one application peak = 948 ± 104 ng/cm^2, 7 applications peak = 1094 ± 25 ng/cm^2), but increasing the application number did result in the SC elimination ½ life being increased from 12.2 ± 4.2 hours (1 application) to 39.5 ± 6.3 hours (7 applications), and in terbinafine being detected for longer periods after therapy (one application detectable up to 72 hours, seven applications detectable up to 168 hours).

The SC concentration of terbinafine (0.33 ng/cm^2 SSB) at 7 days following 7 applications was approx. 100 times higher than the known cidal concentrations for the common dermatophytes causing dermatomycoses. These results suggest that very short courses of therapy (one application daily for seven days or less) of terbinafine 1% cream are likely to be effective in treating superficial dermatomycoses.

URINARY NEOPTERIN IN THE ASSESSMENT OF DISEASE ACTIVITY OF CHRONIC PLAQUE PSORIASIS AND CUTANEOUS T-CELL MALIGNANCIES.

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Urine and plasma neopterin levels reflect T-lymphocyte activation (Huber C et al. Pteridine as a new marker to detect human T-cells activated by allogeneic or modified self major histocompatibility complex (MHC) determinants. J Immunol 1983;130:1047). They have been used to monitor graft rejection, viral infection, autoimmune disease, and various malignancies. However, neopterin has not been investigated in patients with evidence of cutaneous T-cell activation. We have studied its potential for the assessment of disease activity in psoriasis (before and after treatment) and cutaneous T-cell malignancy.

Seven patients with chronic plaque psoriasis of varying severity (2 female, 5 male; age 17-75 years, mean 38) and twelve (7 female, 5 male; age 32-71 years, mean 58) with various cutaneous T-cell malignancies (CTCM) were studied. Fifteen healthy volunteers acted as controls (6 female, 9 male; age 22-52 years, mean 47). Fresh urine samples were collected in antioxidant, frozen immediately and stored at -70°c. Reduced and oxidised forms of urinary neopterin were simultaneously monitored by HPLC with dual electrode and fluorescence detectors. Neopterin:creatinine ratios were used to overcome circadian variation.

Neopterin levels and neopterin:creatinine ratios were significantly elevated in patients with CTCM versus control group ($p<0.05$), but bore no relationship to the clinical stage. Levels were also elevated in chronic plaque psoriasis (p<0 01), and a decrease in neopterin and its creatinine ratios followed treatment (p<0 05 Table 1)

Table 1

	Neopterin Levels ± SD (ng/ml)		
Normals(n=15)	CTCM(n=12)	Ps[PreR$_x$](n=7)	Ps[R$_x$]
567±231	891±916	1364±1006	251±300
		Neopterin/Creatinine ratio	
78±33	301±125	197±285	38±49

Therefore, urinary neopterin levels do not relate to the clinical stage of CTCM, but may be a sensitive marker for disease activity of psoriasis The relationship of neopterin to disease activity of psoriasis and other T-cell dependent skin diseases merits further study.

SO-1100 (SCOTIA EVENING PRIMROSE OIL)-INDUCED REDUCTION IN THE SEVERITY OF RADIATION DAMAGE TO PIG SKIN.

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Anecdotal reports have indicated that skin reactions were less severe than expected in women taking So-1100 when receiving radiotherapy for breast cancer. So-1100 contains 8-10% gamma linolenic acid (GLA), an essential precursor in eicosanoid metabolism. The ability of So-1100 to modify radiation-induced skin damage has been assessed in the pig.

Pigs received daily 3ml of either So-1100 or a placebo, sunflower oil, for 4 weeks prior to and 16 weeks after localised β-irradiation of skin with 25mm diameter strontium-90 plaques. The severity of the erythema in pigs receiving So-1100 was significantly less than in the placebo group; ED_{50} values for bright red erythema were 43.0 ± 2.81 and 31.76 ± 1.39Gy, respectively (p<0.001), a protective factor of 1.3. A similar result was obtained when So-1100 was given after irradiation alone. The duration of moist desquamation was also shorter in the So-1100 pigs i.e. 1.25 ± 0.25 vs 2.9 ± 0.23 weeks (p<0.001). The ED_{50} for moist desquamation was also significantly increased; values were 33.65 ± 0.95 and 30.26 ± 1.22Gy, respectively (p<0.05). Late skin damage, assessed in terms of late erythema or necrosis was also reduced with So-1100, with protective factors of 1.2-1.4. No protection against skin damage was observed if So-1100 was only administered for the 4 weeks prior to irradiation indicating no direct radioprotection effect.

These findings suggest that So-1100 may offer a means of reducing radiation-induced skin damage. So-1100 appears to have no deleterious side-effects. Moreover in vitro data suggest that it may also possess tumouricidal properties, and thus the therapeutic use of So-1100 may represent a safe and valuable method of increasing the therapeutic gain in radiotherapy patients.

COMPARATIVE POTENCY OF BROAD-BAND AND NARROW-BAND PHOTOTHERAPY SOURCES TO INDUCE OEDEMA, SUNBURN CELLS (SBC) AND UROCANIC ACID (UCA) PHOTOISOMERISATION IN HAIRLESS MOUSE SKIN.

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The Philips TL01 narrow-band (311-313nm) fluorescent lamp is efficient in phototherapy for psoriasis and atopic eczema whilst emitting less erythemogenic radiation than conventional broad-band (eg Philips TL12; 280-340nm) sources. We studied the potency of TL01 and TL12 radiation to induce oedema and SBC, and to photoisomerise naturally occuring *trans*-UCA to *cis*-UCA in mouse skin. *cis*-UCA has immunosuppressive properties and is a putative mediator of UVB (280-320nm) induced suppression of skin immune responses.

The dorsal skin of female hairless albino (Skh-1) mice was irradiated with a series of doses (5 mice/dose) ~12.5cm below TL01 or TL12 tubes (irradiance ~1 55 and ~0.96 mW/cm^2 with calibrated meter). 24h later, skinfold thickness (oedema) was measured with a spring-loaded gauge. Interfollicular epidermal SBC were scored in planimeterised H&E stained sections UCA was extracted from epidermis using Finn chambers (filter discs soaked in 0.1M KOH) and %*cis*-UCA quantified by HPLC (Table; mean (SE)).

	~DOSE (mJ/cm2)	%INCREASE OEDEMA	SBC/cm	%*cis*-UCA
	0	0.0 (1.9)	1.9 (0.3)	7.8 (1.6)
TL12	27	9.4 (2.4)	4.0 (0.6)	17.1 (1.3)
	51	18.4 (3.2)	8.5 (1.2)	24.1 (0.3)
	78	30.8 (4.6)	13.7 (2.4)	31.5 (2.1)
TL01	184	4.4 (5.0)	2.5 (0.5)	40.3 (3.4)
	324	14.4 (2.5)	7.0 (0.6)	46.9 (2.3)
	467	23.4 (2.6)	17.7 (1.7)	48.2 (4.9)

For each source there was UV dose dependence for all three responses. Within the dose ranges used, the ratio of TL01:TL12 doses to induce equivalent oedema and SBC was ~6:1. At a given level of oedema or SBC induction, TL01 was always more efficient than TL12 at UCA photoisomerisation. This was particularly apparent at the lowest TL01 dose (184 mJ/cm^2) where negligible skin damage was accompanied by a high (~40%) level of *cis*-UCA induction. This may relate to the high efficacy of TL01 in treating skin conditions with an immunological component whilst causing minimal skin injury.

PUVA ERYTHEMAL SENSITIVITY DEPENDS ON PLASMA PSORALEN CONCENTRATION AND UVA SENSITIVITY.

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We have examined the variation between patients in erythemal sensitivity to PUVA by measuring the UVA and PUVA erythemal response, and plasma 8-methoxypsoralen (8-MOP) concentration in 27 patients about to start PUVA treatment for psoriasis.

10 sites (1 cm diameter) on the back were exposed to increasing doses of UVA (350+/-30 nm; 10-52 J/cm^2) using an irradiation monochromator. At 24h the erythema at each site was measured using a reflectance instrument and the increase in erythema over background was plotted against the log UVA dose. A logit function was fitted to the data to construct a dose-response curve for UVA erythema. Each patient was then given 8-MOP (0.6mg/kg). 2h later blood was taken for HPLC assay of 8-MOP plasma concentration and the UVA phototests were repeated (dose range 0.35-8 J/cm^2). Erythema was measured at 72h and a dose-response curve constructed for PUVA erythema.

The slope of the UVA dose-response curve was positively associated with the slope of the PUVA dose-response curve (p=0.03; Spearman coefficient of rank correlation). We confirmed our previous findings (McLelland et al, Br J Dermatol 1990;123:823) of a significant correlation between the slope of the PUVA dose-response curve and the log plasma 8-MOP concentration (p=0.02). By allowing for the confounding effect of plasma 8-MOP concentration on the slope of the PUVA dose-response curve a highly significant correlation (p<0.005) was obtained between this and the slope of the UVA dose-response curve.

These results suggest a common pathway in the vascular response induced by UVA radiation with or without the presence of 8-MOP and show that erythemal sensitivity to PUVA, measured as the slope of the dose-response curve, is related to both plasma 8-MOP concentration and inherent sensitivity to UVA.

PHOTOADAPTATION AFTER 5 DAYS OF PHOTOTHERAPY IS DUE TO INCREASE IN MELANIN PIGMENTATION RATHER THAN EPIDERMAL THICKENING.

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The differences in the absorbance of protein and melanin at 280 and 320nm were used to examine the relative contribution of tanning and epidermal thickening to the change in erythemal sensitivity of the skin following 5 days phototherapy.

A reflectance instrument was used to measure the 24h erythemal response on one foream to a series of UV doses at 280 and 320nm. The other arm was exposed to 50% of the minimal erythema dose on 5 consecutive days using fluorescent UV lamps (peak emission 325nm). 5 days after the last exposure the treated arm was phototested at 280 and 320nm as above. For each subject the increase in erythema (ΔE) at each site was plotted against the log of the dose at 280 and 320nm before and after phototherapy. A logit function was fitted to the data to construct a dose-response curve and the dose of radiation required to achieve a ΔE of 0.1 (moderate erythma) was calculated.

Skin type	Subject	Dose required for ΔE of 0.1 280 ± 5nm (mJcm^{-2}) control	 irradiated	 ratio	 320 ± 10nm (Jcm^{-2}) control	 irradiated	 ratio
I	1	230	329	1.4	5.2	6.3	1.2
II	2	288	512	1.8	4.9	8.6	1.8
II	3	278	256	0.9	6.7	6.1	0.9
III	4	269	>634	>2.4	6.6	9.6	1.5
III	5	330	740	2.3	6.2	8.6	1.4
IV	6	200	381	1.9	5.6	10.0	1.8
IV	7	313	571	1.8	6.5	11.0	1.7
IV	8	299	>634	>2.4	7.7	16.1	2.4
			Median	1.8		Median	1.6

The similar ratios at both wavelengths show that only pigmentation has altered as epidermal thickening would have caused a much greater change in sensitivity at 280nm. In skin types I to IV, 5 days of UV exposure using therapeutic doses causes a 1.6 to 1.8 fold decrease in erythemal sensitivity. This adaptation is due to increased pigmentation rather than epidermal thickening.

65 KILODALTON HEAT SHOCK PROTEIN EXPRESSION IN EXPERIMENTALLY-INDUCED POLYMORPHIC LIGHT ERUPTION.

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Although the cause of polymorphic light eruption (PLE) remains unknown, recent research suggests a cellular inflammatory response to an as yet unidentified UV-induced or promoted antigen in its pathogenesis[1]. Heat shock, or stress proteins (HSP) are candidates for such an antigen. Stress proteins are highly conserved between species and are produced by cells in response to certain noxious stimuli; they may also be recognised by the cellular immune system. The monoclonal antibody ML-30, originally raised against m.leprae HSP 65, also recognises human 65 kDa HSP. We investigated the expression of HSP 65 immunoreactivity of skin biopsy sections from experimentally-induced PLE lesions, using ML-30 at a dilution of 1/250 and an indirect immunoperoxidase technique.

In each subject the 24 hour minimal erythema dose of solar simulated radiation (Kratos 2500W, xenon arc filtered, model no. LM 152N/3SS) was determined. 4 x 4 cm. area of skin previously affected by ple was then subjected to 70% minimal erythema dose, in order to induce PLE lesions. Biopsies were performed at 0 (n=3) hours and 1 (n=2), 5 (n=3), 24 (n=3), 72 (n=2) and 144 (n=1) hours post-irradiation. 7 subjects with PLE were recruited, but biopsies were only performed on a limited number of occasions for each subject to avoid multiple scars. A similar irradiation procedure was performed on 2 normal subjects, and biopsies were taken at 0 and 24 hrs. Labelling of skin biopsy sections by ML-30 antibody was assessed in a blind fashion.

In PLE subjects clinical inflammation was noted at 5 hours post-irradiation, with subsequent evolution of PLE-like lesions; this was sustained through to 144 hours. An increase in labelling of ML-30 antibody in epidermal keratinocyte cytoplasm and endothelial cells was recognisable from 1 hour post-irradiation, and in dermal dendritic cells from 5 hours. This increase in labelling was sustained through to 144 hours. Inflammatory cell infiltration was noted from 5 hours, and the mononuclear cell component was markedly labelled. In normal subjects neither clinical nor histological features of inflammation were noted in response to irradiation, and there was no increase in HSP 65 labelling.

In experimentally induced PLE, HSP 65 labelling was therefore raised in both dermis and epidermis. It is of interest that the cellular immune response in induced-PLE has been compared to a ppd reaction[1], as HSP 65 is a major mycobacterial antigen. The role of stress proteins in the induction of polymorphic light eruption warrants further investigation.

1) PG Norris, J Morris, DM McGibbon Br.J.Dermatol., 1989; **120**: 173-183.

LYMPHOCYTE ATTRACTANT ACTIVITY IN SUCTION BLISTER FLUID (SBF) SAMPLES FROM POLYMORPHIC LIGHT ERUPTION (PLE) AND CHRONIC ACTINIC DERMATITIS (CAD).

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PLE and CAD are characterized by a lymphocyte-rich infiltrate. In order to study the release of lymphocyte attractant cytokines in these disorders, lesions of PLE (5 patients) and CAD (4 patients) were induced by a single exposure to solar simulated radiation (Kratos Xenon Arc 2500 W). Suction blisters were raised on these sites 1-3 h and 23-25 h post-irradiation and on adjacent uninvolved skin prior to irradiation. Lymphocyte migration in response to >3 kiloDalton ultrafiltration fractions was measured using a 48-well microassay as described (Bacon KB et al, Br J Pharmacol 1988; 95: 966-74). As previously reported (Bacon KB et al, Eur J Immunol 1990; 20: 565-71), samples from clinically normal, control skin induced substantial, dilution-related lymphocyte migration (maximum migration index [MMI] 2.1 ± 0.2 for PLE; 2.2 ± 0.1 for CAD; means ± sem). In comparison with these control results, there was no significant change in dose-response curves generated by samples following irradiation; the MMI values for PLE samples at 1-3 h and 23-25 h were 2.0 ± 0.2 and 2.3 ± 0.1 respectively, and for CAD samples 2.3 ± 0.2 and 2.5 ± 0.1 respectively. These MMI values also occurred at similar dilutions to those obtained with the control samples. The use of neutralizing antibodies (NAb) suggested a changing profile of recoverable lymphocyte attractant cytokines following irradiation. Thus, pre-incubation of lymphocytes with NAb reduced MMI values induced by control PLE SBF samples to 1.6 ± 0.1 and 1.6 ± 0.2 for anti-IL-1 alpha and beta respectively, and by control CAD SBF samples to 1.1 ± 0.1 and 1.1 ± 0.1 respectively. In contrast, NAb against IL-6 and IL-8 had little or no effect on the activity in both PLE and CAD control samples. In PLE 1-3 h post-irradiation SBF-induced lymphocyte migration was partially inhibited by NAb to IL-6 (MMI 1.4 ± 0.2), and by NAb to IL-1 alpha (MMI 1.3 ± 0.1) and IL-1 beta (MMI 1.3 ± 0.1) and at 23-25 h also by neutralization of IL-8 (MMI 1.5 ± 0.1). In CAD 1-3 h post-irradiation SBF-induced lymphocyte migration in the presence of NAb was similar to control, but at 23-25 h partial inhibition of migration by IL-6 (MMI 1.4 ± 0.1) but not IL-8 (MMI 2.0 ± 0.1) NAb was demonstrated.

These findings confirm earlier results showing that normal skin contains biologically active IL-1-like material, which may play a role in physiological lymphocyte trafficking. In post-irradiation SBF, inhibition of activity by NAb suggested the additional presence of IL-6- and IL-8-like material in PLE, and IL-6 but not IL-8 in CAD. These cytokines may therefore play a role in the induction of lymphocyte infiltration in these photodermatoses. Alternatively, the finding that the stimulation of overall lymphocyte locomotor activity by post-irradiation samples was no greater than controls, may suggest that lymphocyte adhesion molecule expression or the enhanced locomotor responsiveness of activated lymphocytes may be more relevant than the generation of fresh attractant gradients, in the pathogenesis of the lymphocyte infiltrates.

DERMAL PAPILLA CELLS FROM HUMAN PUBIC HAIR FOLLICLES DO NOT METABOLISE TESTOSTERONE TO 5α-DIHYDROTESTOSTERONE IN CULTURE.

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Androgens regulate many human hair follicles but in 5α-reductase deficiency only pubic, axillary and scalp hair growth occurs; this suggests that 5α-dihydrotestosterone (DHT) is the active intracellular androgen except in axillary and pubic follicles. Since the dermal papilla plays a major regulatory role in the follicle and may be the site of androgen action, we have investigated androgen metabolism in cultured dermal papilla cells from pubis.

After 24h in serum-free E199 medium, 100mm dishes of confluent cells were incubated for 2h with 5nM[1,2,6,7-^{3}H] testosterone. Media was collected, the cells washed (x4) with saline and extracted with chloroform:methanol (2:1), before unlabelled and ^{14}C-steroids were added and the extracts analysed by thin layer chromatography; steroid identity was confirmed by recrystallisation to constant ^{3}H:^{14}C ratio.

In both cells and media (4 cell lines) the major steroid identified was testosterone, but significant amounts of androstenedione were present indicating 17β-hydroxysteroid dehydrogenase activity; androstanedione was also identified within the cells, but not DHT.

These results contrast with our earlier observation of DHT in beard cells and confirm that androgen metabolism in cultured dermal papilla cells reflects the parent follicle's ability to respond to androgen in the absence of 5α-reductase. This supports the hypothesis that androgens act on hair follicles via the dermal papilla and the importance of cultured dermal papilla cells as a model system for studies on androgen action.

ANDROGENS STIMULATE THE SECRETION OF MITOGENIC SUBSTANCES(S) BY DERMAL PAPILLA CELLS CULTURED FROM BEARD BUT NOT SCALP FOLLICLES

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Androgens stimulate many hair follicles, including beard, but are unnecessary for normal scalp hair growth Since the dermal papilla regulates many aspects of follicular activity and may be the site of androgen action, the production of androgen-regulated mitogenic factors by primary lines of cultured dermal papilla cells from beard (n=4) and non-balding scalp (n=7) follicles has been investigated.

Confluent dermal papilla cells were incubated for 24h in serum-free E199 media with or without testosterone (10^{-10}-10^{-5}M) Each conditioned medium was assayed individually with, or without, the addition of testosterone (10^{-10}-10^{-5}M) by measuring ^{3}H-thymidine incorporation in dermal papilla cells as described previously (Randall et al, (1990), Br.J.Derm 123, 815)

Testosterone increased the mitogenic effect of beard cell media on beard cells (n=4) in a dose-responsive manner with maximal effect at 10^{-8}M ($p<0.05$), but caused no response in scalp cells (n=4). It had no effect on the mitogenic potential of scalp cell media regardless of target cell Adding testosterone to media conditioned by beard cells (n=4) in the absence of androgen also had no effect.

These results are consistent with the theory that androgens regulate the hair follicle via the dermal papilla. They indicate not only that androgens stimulate mitogenic factors solely by cells from androgen-dependent follicles, but also that the ability to respond to these is only found in androgen-dependent cells. Although the obvious target for such factors would be the follicular epithelial cells, dermal papillae from bigger follicles are larger and this may involve dermal papilla cell growth Further studies of such factor(s) should improve our understanding of how androgens regulate human hair growth.

THE CULTURE OF DERMAL PAPILLA CELLS FROM THE MANE AND FLANK OF THE RED DEER: A COMPARISON OF GROWTH AND ANDROGEN RECEPTOR CONTENT

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The adult male red deer displays a mane during the breeding season, presumably in response to increased levels of testosterone. The mane parallels the human beard but in contrast only grows for a short period, so this animal may provide a good model for human studies of hair growth. We have compared the growth and androgen receptor content of cultured dermal papilla cells from mane and flank follicles.

Cells were grown in medium E199 with 10% foetal bovine serum in 35mm dishes and counted over a 12 day period, or 100mm dishes and incubated with ^{3}H-mibolerone (0.05-10nM) + 1000x excess triamcinolone acetonide ± 100x excess 5α-dihydrotestosterone following a 24h incubation in serum-free medium. These were washed and radioactive uptake estimated after extraction with chloroform:methanol.

There was no difference in the rate of growth of cells derived from mane (n=2) or flank (n=2) follicles. However, specific androgen receptors were saturable at about 1nM in mane cells; mean Kd 0.77nmol, mean Bmax 0.04 fmol/10^4 cells; 11 fmol/mg protein from Scatchard analysis, but little specific binding was detected in corresponding flank cells. Specific androgen receptors were also present in dermal fibroblasts from mane skin (n=1), Kd 0.37nmol, but the level was lower than that of the dermal papilla cells (Bmax 0.02 fmol/10^4 cells; 3.33 fmol/mg protein).

These results support the theory that androgens may act on the hair follicle via the dermal papilla.

FURTHER INVESTIGATION OF ETRETINATE ALOPECIA.

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It has previously been demonstrated that the alopecia induced by etretinate does not conform to classical descriptions of either anagen or telogen effluvium. (Berth-Jones J, Hutchinson PE. A study of etretinate alopecia. Br J Dermatol 1990; 122: 751-5). The aim of this study was to test the hypothesis that the hair effect of this drug is due to an ongoing general reduction in the duration of anagen, and an arrest of anagen onset.

Fifteen subjects were investigated while receiving etretinate for 6 months at a mean dose of 0.78 mg/kg/day. Parameters studied included 5-day shed hair counts at baseline and at 6-week intervals, pluck telogen counts at baseline and after 12 and 24 weeks, and hair density measured by shaving an area of 0.5 cm^2 and counting the cut hairs at baseline, 6 and 18 weeks. In addition, the proportion of short anagen hairs of less than 5cm length was counted in each plucked and shaved hair sample.

Mean shed rates increased steadily from 31 hairs/day at baseline to 138 by 24 weeks. Mean hair density fell from 220/cm^2 to 158 by 18 weeks. Mean pluck telogen counts rose from 11% at baseline to 22% at 12 weeks and 23% at 24 weeks. The mean proportion of short anagen hair changed very little throughout the study, this was 16% at baseline, and 17% at six months.

These data support our hypothesis that etretinate alopecia is caused by the combination of a reduction in the duration of anagen, and a partial arrest in anagen onset, i.e. a prolongation of telogen.

IMMUNOHISTOCHEMICAL LOCALISATION OF ANDROGEN RECEPTORS IN ACNE PATIENTS WITH THE MONOCLONAL ANTIBODY F39.4.1.

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Acne vulgaris is a follicular disease commonly found in adolescents. It is characterised by increase sebum excretion from the sebaceous glands and hypercornification of the duct. The seborrhoea is caused by the development of the sebaceous glands at puberty under hormonal influences, in particular the androgens. However, there is at present no direct evidence linking hypercornification of the duct with androgens. The recent development of monoclonal antibodies to androgen receptors provides a useful tool to investigate the expression of these receptors in tissues and their use should throw some light on the role androgens play in hypercornification.

Cryostat sections (6um) of biopsies obtained from the interscapular region of acne patients (n=15) and normal volunteers (n=5) were stained with anti-androgen monoclonal F39.4.1 (a generous gift from Dr. Netty Zegers, TNO Medical Biological Laboratory, The Netherlands). The data were expressed as the number of positive nuclei in a given area as a percentage of the total number of nuclei in that area. Androgen receptors were present in about 59% of sebocytes from human sebaceous glands from normal follicles and 62% of sebocytes from acne patients and in about 45% of the atrophied sebaceous glands associated with acne lesions. For the number of sections investigated so far, the values were not significantly different. In all cases the receptors were localised exclusively in the nuclei predominantly of the basal sebocytes. The keratinocytes of the duct and epidermis were negative with respect to androgen receptor staining.

This appears to be the first report confirming the presence of androgen receptors in human sebaceous glands by immunohistochemistry. However, failure to detect positively staining cells in the duct seems to suggest it is not subject to androgen control. Other factors, such as retinoic acid or linoleic acid concentration in sebum may be more influential in causing hypercornification in acne.

IMMUNOHISTOCHEMISTRY OF STEROID RECEPTORS IN VAGINA AND VULVAL SKIN. EVIDENCE FOR LOSS OF ANDROGEN AND OESTROGEN RECEPTORS IN THE LESIONAL EPIDERMIS OF VULVAL LICHEN SCLEROSUS.

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Lichen sclerosus et atrophicus (LSA) most frequently affects the vulval skin of postmenopausal women. Topical androgens, oestrogens and progesterone have been used in treatment. To understand the role of sex-steroids in LSA, receptors for androgens (AR), oestrogens (ER) and progesterone (PR) in lesional and perilesional vulval skin of 19 women with LSA were compared with receptors in control vulval skin (25 specimens), suprapubic skin (8 specimens) and vagina (13 specimens) by immunoperoxidase staining with specific receptor antibodies PR were present in vagina but not in skin In controls, ER were present in basal and suprabasal vaginal epithelium and stromal fibroblasts but, in skin, were restricted to epidermal basal cells and occasional dermal fibroblasts. Frequency and intensity of ER staining were highest in vagina and lowest in suprapubic skin. AR were present in vaginal epithelial basal cells and stromal fibroblasts. In skin, AR were present in epidermal basal and suprabasal cells, in dermal fibroblasts and in pilosebaceous units. Frequency and intensity of epidermal AR staining were greatest in vulval skin. Differences in AR or ER between pre- and postmenopausal controls were not seen. AR and ER staining was greatly reduced in epidermis of vulval LSA lesions (AR: 1/19 positive ER 0/19 positive) compared with control epidermis (AR. 24/25 positive. ER: 11/20 positive). The difference was not seen in perilesional epidermis This study has identified the vulval epidermis as a potential target of androgen and oestrogen action Differences in receptors were seen between vagina, vulval and suprapubic skin. The apparent loss of AR and ER from the lesional epidermis may be of significance in the aetiology and treatment of vulval LSA.

CLASS I AND CLASS II HLA ANTIGENS IN CHRONIC BULLOUS DISEASE OF CHILDHOOD.

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Chronic bullous disease of childhood (CBDC) is an uncommon blistering disease of childhood characterised by sub epidermal blisters, with a linear band of IgA on direct immunofluorescence. The aetiology of the disease is unknown but it is thought to have an autoimmune basis. Previous studies have shown an increased incidence of HLA B8. The aim of this study was to determine the frequency of Class II HLA antigens which have not previously been studied, and to identify any correlation with disease activity.

21 patients (14 male and 7 female), mean age of onset 3.6 years. in whom CBDC was diagnosed by clinical and direct immunofluorescence criteria were interviewed about the clinical course of their disease using a standard questionnaire. Blood was taken and HLA typing was performed using standard laboratory techniques. Gene frequencies in the population were calculated from a random panel of healthy controls, and the comparisons analysed using Chi square tests.

A significant increase in HLA B8, DR3 and DQw2 was found. HLA B8 was found in 68% of patients (Normal 19.2%, $p<0.01$). The incidence of DR3 was 73% (Normal 24%, $p<0.01$), and for DQw2 was 73% (normal 36%,$p<0.05$). Comparison between HLA determinations and clinical findings in our patients showed no correlation between HLA and the course of the disease.

Our findings lend support to an immunogenetic basis to the disease, and may explain the increased incidence of circulating anti basement membrane zone antibodies.

EXPRESSION OF CELL SURFACE ADHESION MOLECULES AND COMPONENTS OF ADHERENS JUNCTIONS IN DARIER'S DISEASE AND HAILEY-HAILEY DISEASE

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Darier's disease [DD] and Hailey-Hailey disease [HH] are dominantly inherited dermatoses of uncertain pathogenesis which are characterised by the presence of suprabasal keratinocyte acantholysis. Ultrastructural studies have revealed breakdown in the desmosome-keratin filament complexes in both diseases In previous studies we have shown that there is normal expression of the major components of the desmosomal junctions in uninvolved skin but internalisation of desmosomal components by the acantholytic cells. Keratinocyte adhesion is dependent on the presence of adherens junctions and non-junctional adhesion molecules as well as desmosomes In this study we have used antibodies to a number of adhesion molecules to explore their expression in DD and HH The lectins, PNA and SBA, and sera from patients with the autoimmune diseases pemphigus vulgaris [PV] or pemphigus foliaceous [PF] were used to examine surface glycoproteins

There was normal expression of plakoglobin,desmoyokin, desmocalmin and E-cadherin at the periphery of the keratinocytes in uninvoved skin PNA, SBA and pemphigus sera also bound to the periphery of the keratinocytes in uninvolved skin Desmoyokin and desmocalmin [desmosomal components] were expressed diffusely by the acantholytic cells in involved skin in both diseases. The staining was similar to that seen for desmoplakin in our previous study There was bright focal intracellular expression of plakoglobin [found in desmosomes and adherens junctions] in the acantholytic cells in HH but not in DD E-cadherin [probably in adherens junctions] expression was weak or absent at the periphery of some acantholytic cells. PNA and SBA binding was also reduced around some acantholytic cells Pemphigus antibodies did not bind to the acantholytic cells in involved skin in either disease

The expression of plakoglobin by acantholytic cells was different in HH and DD and this may have relevance to the pathogenesis of the diseases Loss of some adhesion molecules but not others suggests that there is more than one mechanism for breaking down junctions when cells separate and may be secondary to proteolysis

Antibodies to desmoyokin, desmocalmin and E-cadherin were kindly provided by Prof S Tsukita, Okazaki, Japan. This work was supported by the SDRF and Oxford District Research Fund

LEUKOCYTE ADHESION TO PSORIATIC EPIDERMIS.

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T-lymphocytes and neutrophils accumulate in psoriatic epidermis. To determine whether epidermis plays an active role in this process through the production of cellular adhesion factors, leukocyte adherence to lesional psoriasis (n = 6) was compared to normal skin (n = 6) in a modified frozen section adhesion assay.

Lymphocyte and neutrophil suspensions were prepared by standard Ficoll-Hypaque techniques from peripheral blood of normal volunteers. Lymphocytes were activated with TPA (10nM, 30mins.), suspended in RPMI and $1x10^6$ cells (neutrophils or lymphocytes) overlaid on to glutaraldehyde-fixed 8um cryostat sections of skin. Sections were then incubated for 45 minutes at 37°, washed and stained with toluidine blue to allow quantification of adherent cells

Lymphocyte adhesion to epidermis was significant greater in psoriasis, mean = 13.40 (sem ± 1.1) lymphocytes per high power field compared to normal skin, mean = 1 22 (sem ± 0.23), $p < 0.001$ Likewise, neutrophils adhered more prominently to psoriatic epidermis, mean = 60.7 (sem ± 22), compared to normal skin, mean = 4.67 (sem ± 3.3), $p < 0.05$ Whereas neutrophils were uniformly distributed throughout the epidermis, lymphocyte adhesion was most prominent at the tips of dermal papillae, corresponding to areas of maximal ICAM-1 expression Both neutrophils and lymphocytes adhered to dermal papillary vascular endothelium.

These studies provide functional data that psoriatic epidermal cells are actively involved in leukocyte adherence. The distribution of adhesion suggests that both ICAM-1 dependent and independent mechanisms are involved.

A QUANTITATIVE ASSESSMENT OF INTEGRIN EXPRESSION IN NORMAL AND PSORIATIC EPIDERMIS.

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The altered epidermal differentiation and increased epidermal turnover in psoriasis both involve changes in intercellular adhesion. The pattern of staining of α and ß-1 integrins has recently been characterised for normal epidermis (Eberhard Klein et al. Br J Dermatol. 1990;123:171-8). Little is known about their distribution in disease states. A quantitative immunohistochemical comparison of the expression of the integrin family of adhesion molecules in normal (N=7) and psoriatic (N=12) buttock skin biopsies. Antibody labelling integrin epitopes was disclosed using an avidin-biotin labelling technique.

Keratinocytes expressed subunits ß-1, ß-4, α-2, α-3 and α-6 in characteristic patterns in normal and psoriatic epidermis. Large epidermal dendritic cells (antigen presenting cells) express ß-2 integrin. These are rarely seen in normal skin but were found to be present in large numbers in all the psoriatic biopsies examined. The epidermis showed a 73% increase in mean ß-1 staining in psoriatic biopsies as compared with normal ($p<0.002$). α-3 and α-6 showed non significant rises in staining (both 23%). α-2 and ß-4 staining was not altered.

The differential increase in ß-1 integrin over ß-4 integrin in the epidermis indicates that keratinocytes undergo a change in phenotype rather than simply retaining basal characteristics in a failure of maturation. The increase in ß-2 positivity is consistent with deranged antigen handling in the epidermis of psoriasis.

MONOCLONAL ANTIBODIES IN CUTANEOUS T CELL LYMPHOMA.

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We have assessed the value of a panel of 7 commercially available antibodies directed against specific sites of the variable region of the T cell receptor (TCR) in the differentiation of cutaneous T cell lymphoma (CTCL, 15 cases) from reactive dermatitis (5 cases). Strong positive staining with only one of these antibodies would be an indication of monoclonality. In addition, we have used antibody Ki 67, which identifies cycling cells, to assess the presence of cycling intraepidermal T cells as a marker of CTCL. The antibodies which were used were αV2a, βV5a, βV5b, βV6a, βV8a, βV12a and V$\alpha\beta$a on frozen sections at 1/100 dilution in an indirect biotin avidin immunoperoxidase technique.

Six CTCL cases showed strong staining of the majority of lymphocytes with antibodies βV8a and βV12a, and a further 5 showed strong staining with one of these alone. No other TCR antibody showed staining of a majority of T cells in CTCL cases, and none of the reactive dermatitis cases showed staining of the majority of T cells with only one of these antibodies. 8/15 (53%) CTCL cases showed intraepidermal Ki 67 positive T cells, compared with 1/5 (20%) of dermatitis cases.

Our results show that 11/15 (73%) of CTCL cases can be identified using 2 monoclonal antibodies to the TCR V region, but the presence of dual staining does not support monoclonality. They suggest that a limited repertoire of TCR rearrangements may operate in CTCL, and that Ki 67 intraepidermal T cells are useful but neither totally sensitive nor specific for CTCL.

EFFECTS OF α-MSH AND Ca^{2+} ON THE ATTACHMENT OF HUMAN MELANOCYTES TO LAMININ AND FIBRONECTIN.

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The ability of cells to interact with laminin (LM) and fibronectin (FN) is considered to regulate their motility, differentiation and growth. α-Melanocyte stimulating hormone (α-MSH) and Ca^{2+} ions may also influence pigment cell properties thus their effects on the attachment of human melanocytes cultured from pre-pubertal foreskins were studied.

Attachment of ^{3}H-thymidine-labelled cells to tissue culture wells coated with bovine serum albumin (BSA), LM, FN or LM+FN was performed in at least triplicate: standard deviations were generally within 10% of means.

In DMEM (1.8mM $CaCl_2$), cells from the two cultures, M6 and M81, attached to all substrata but preferentially to FN: $p<0.05$ relative to plastic in both cases. For M6, attachment to all substrata was shown to increase with time up to 2 h. Treatment of the cultures with 10^{-9}M α-MSH for 72 h caused an increase in attachment of M6 to LM and FN while retaining the propensity for FN. α-MSH had a similar effect on the attachment properties of M81. The effect of Ca^{2+} in combination with α-MSH was investigated using M81 cells. In the absence of α-MSH and Ca^{2+} (tris-buffered saline), attachment was poor but the propensity to FN remained. 10^{-9}M α-MSH, in the absence of Ca^{2+}, did not affect attachment to FN but increased adhesion to LM and LM+FN 2-fold in each case, resulting in a preference for the LM+FN substratum. Tris-buffered 1mM $CaCl_2$, in the absence of α-MSH, increased attachment to LM+FN 1.5-fold, to FN 2-fold and to LM 5-fold, thus reducing the differential in attachment to LM and to FN seen in the absence of Ca^{2+}. In 1mM $CaCl_2$ with α-MSH, adhesion to LM+FN and to FN both increased 3-fold and to LM 8-fold so that final attachment to the latter two substrata was similar. In conclusion, this indicates that: 1) while human melanocytes can attach to both LM and FN, the pattern of attachment appears to be influenced by both Ca^{2+} and α-MSH; 2) the action of α-MSH on attachment to FN requires Ca^{2+} while the effect on attachment to LM and LM+FN does not; 3) Ca^{2+} and α-MSH may, however, act synergistically to influence the attachment of human melanocytes.

Posters

THE OBJECTIVE ASSESSMENT OF NAIL BRITTLENESS.

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Nail brittleness has functional and cosmetic implications [Kechijian P (1985): Brittle Fingernails. *Dermatologic Clinics* 3(3) pp421-429]. It results from both chemical and environmental insults and occurs in many skin and systemic diseases. Although it is very common there is no definition of nail brittleness, and no method of measurement.

We have designed a method for the in vivo measurement of some parameters of nail failure under controlled conditions. We have constructed a device to implement and test this method on normal, experimentally altered and diseased nails.

The device is best described as a modified punch and die, where the subject places a protruding distal nail onto the die, and a blunt pin type punch of much smaller diameter than the die is placed onto the top surface of the nail. The punch is pressed until it breaks through the nail while the force applied and the position of the punch are recorded simultaneously.

Hard and brittle nails require greater force to puncture and allow less compression of the nail before failure, while softer or more pliable nails allow more movement of the punch before they are punctured. We have proposed two indices to describe such behaviour: a Malleability Index (MI) and a Failure Index (FI). These relate the breaking strength and the 'give' before failure. The table below describes how they change when typical nails are softened by 15 mins water immersion.

	DRY		WET	
NAIL	MI	FI	MI	FI
THUMB	460	0.0133	304	0.026
MIDDLE	292	0.0124	168	0.0139
LITTLE	287	0.0123	129	0.0235

The technique could be used to measure the degree of nail brittleness in patients with clinically brittle nails; to assess the possible relationship between certain medical and dermatological conditions and nail brittleness; and as a means of assessing the effects of systemic or local treatment on nails.

RELAPSE MARKERS IN PSORIATIC EPIDERMIS AFTER SYSTEMIC CYCLOSPORIN THERAPY.

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The adhesion molecules ß1 integrin (VLA common subunit, CD29), ß2 integrin (CD18), and intercellular adhesion molecule-1 (ICAM-1, CD54) are essential to the intimate co-operation of antigen presenting cells (APCs), T cells and keratinocytes. The efficacy of cyclosporin in psoriasis may be partly explained by altered integrin expression. A quantitatve immunohistochemical assessment of the effect of low dose cyclosporin (2.5 or 5.0 mg/kg/day) on the expression of ß1-integrin, ß2-integrin and ICAM was performed on the epidermis of 14 patients with chronic plaque psoriasis. Punch biopsies (6mm) were taken from involved and uninvolved buttock skin before starting and on stopping a three month course of cyclosporin. Staining levels were compared with clinical response as assessed by the PASI score.

There was an excellent response to treatment with a fall in mean PASI score from 13 at initiation of therapy to 2.7 after three months on therapy ($p<0.00001$). One month after stopping therapy nine patients had relapsed by greater than 50% compared to their PASI score at the termination of therapy. ß2 and ICAM staining in uninvolved skin usually disappeared after cyclosporin therapy. In involved skin the overall ß1-integrin and ICAM expression on keratinocytes were not altered by therapy. In two patients who showed an excellent clinical response to cyclosporin keratinocyte ICAM expression was greater following cyclosporin than before treatment. There was a significant decrease of 70% in the mean level of ß2 positive large dendritic cells (APCs) within the epidermis ($p<0.008$). ß2-integrin was not expressed by keratinocytes. There was a strong correlation between ß2 expression and PASI score after three months on cyclosporin (r_p=0.812; $p<0.02$) and one month off therapy (r_p=0.754; $p<0.02$).

These results indicate that the number of ß2 integrin positive large dendritic epidermal cells (APCs) is a marker of susequent clinical relapse of chronic plaque psoriasis following the withdrawal of low dose cyclosporin.

DOSE-RESPONSE EFFECTS OF INTRADERMAL KALLIDIN AND BRADYKININ IN NORMAL SUBJECTS AND PATIENTS WITH PSORIASIS.

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With the recent development of selective drugs acting on the kinin system and the identification of a kallikrein-like enzyme from psoriatic blister fluid (Nicholls et al, Br J Dermatol, 1990; 123; 819) there is now much interest in the possible role of kinins in psoriasis. We have examined the time course of the inflammatory responses to intradermal kallidin (lyse-bradykinin) and bradykinin in normal volunteers and have compared the dose response effect of these agents in normal and psoriatic volunteers.

Initially, normal subjects (N=5) received coded intradermal injections of 50 ul.N. saline containing kallidin or bradykinin (0.1, 0.5, 1.0, & 5 0ug). Weal volume, area and flare area were calculated at 5, 15, 30 & 60 minutes, by measuring two perpendicular diameters and change in skin fold thickness. Weal and flare measurements were subsequently made at 15 & 5 minutes, the times of maximal response.

Psoriatic and normal subjects (N=12) were given intradermal injections into clinically normal forearm skin of kallidin (0.1 & 1.0ug) and bradykinin (0.1, 0 5 & 1.0ug) using histamine (0.5ug) and N. saline as controls.

The dose-response effect of kallidin on weal and flare responses in human skin have been established in this study and compared with those of bradykinin There was wide inter-individual variability for both agents and, although mean responses to the highest doses of kallidin and bradykinin were decreased in psoriatic skin, no significant differences were found between the psoriatic and normal group for either kallidin, bradykinin or histamine.

Hence there do not appear to be any obvious altered vascular responses to kallidin or bradykinin in patients with psoriasis, despite the fact that kinins may be generated in psoriatic tissue.

EFFECTS OF ORAL DOXEPIN ON PLATELET ACTIVATING FACTOR (PAF) - INDUCED INFLAMMATORY RESPONSES IN HUMAN SKIN.

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Intradermal injection of platelet activating factor (PAF, Paf-acether, AGEPC) in human skin induces weal and flare responses, which seem to be mediated in part by histamine release (Archer et al. Br J Pharmacol,1985,85:109-113). Based on the treatment of cold urticaria, doxepin has been suggested to have PAF antagonist activities (Grandel et al., N Engl J Med 1985;313:405-9) in addition to being an H_1- and H_2-receptor antagonist. The aim of the present study was to compare the effects of oral doxepin on PAF-induced weal and flare responses in normal volunteers with those of terfenadine (an H_1-antagonist) and cimetidine (an H_2- antagonist).

In an initial study, 6 normal subjects received 50 ul intradermal injections of PAF (10ng, 30ng, 100ng, 200ng) and lyso-PAF (100ng) and saline as controls before and after treatment with oral doxepin (10mg bd for 3 days). To minimize bias solutions were coded and randomly assigned to marked sites on the flexor aspect of the forearms. Weal volume and flare area responses were calculated 15 and 5 minutes after injection, by measuring 2 perpendicular diameters and change in skinfold thickness. In a further study, 6 normal subjects received PAF (100ng, 200ng), histamine (0 5ug), codeine (10ug), a histamine liberator, and lyso-PAF (100ng) before and after oral doxepin, terfenadine (60mg bd for 3 days) and cimetidine (400mg bd for 3 days), on these occasions weal responses being assessed by change in skinfold thickness.

In the initial study, doxepin significantly reduced PAF-induced flare area responses at all doses of PAF and weal volume responses at the two higher doses ($P<0.01$) In the second study, as expected, doxepin and terfenadine significantly decreased weal and flare responses to both histamine and codeine ($P<0.01$), but these responses did not alter significantly following cimetidine Both doxepin and terfenadine significantly attenuated PAF-induced weal and flare responses to a similar extent ($P<0.01$)

The study shows that doxepin inhibits PAF-induced inflammatory responses in human skin However, in view of the significant effects of terfenadine, the effects of doxepin on cutaneous PAF-induced responses may be due to its known antihistaminic activity and we cannot comment on any specific PAF-antagonist effects of this drug.

A STUDY OF THE EFFECTS OF DITHRANOL ON THE 12-LIPOXYGENASE PATHWAY IN WASHED PLATELETS AND NEUTROPHILS.

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Despite its efficacy in the treatment of psoriasis the exact mode of action of dithranol is yet to be determined. In the psoriatic plaque a prominent abnormality of eicosanoid metabolism is an increase in epidermal 12-HETE. We have therefore studied the *in vitro* effect of dithranol on the conversion of arachidonic acid to 12(S)HETE in human platelets and its further metabolism as determined by the ω-oxidation of 12(S)HETE to 12,20 DiHETE in unstimulated neutrophils.

Washed platelets (300×10^6 cells/ml) were prepared by centrifugation, and incubated for 2 minutes with a blank control or a range of concentrations of dithranol (as shown below) prior to the addition of arachidonic acid (6.25µM). After a further 5 minutes, the reaction was terminated by mixing the aliquot with an equivalent volume of HPLC buffer (78% methanol), plunging into ice and microcentrifugation at 13000 rpm to remove the cells. The 12(S)HETE produced was measured by RP-HPLC and the inhibition calculated as a percentage of the control.

Neutrophils (5×10^6 cells/ml) were incubated for 2 minutes with the same range of concentrations of dithranol or control prior to the addition of 12(S)HETE (6.2µM). After further incubation for 10 minutes the reaction was terminated as described above and the12,20 Di-HETE produced was measured by RP-HPLC. Inhibition was calculated as a percentage of the control.

Dithranol had a marked inhibitory effect on 12(S)HETE production. The following values were obtained (n=5)

Dithranol conc. µM	1	3.3	10	33	100
% of control	84	91	54	15	0 6
SE	10	10	17	10	0.5

Dithranol had a lesser effect on the breakdown of 12(S)HETE to 12,20 DiHETE through the ω-oxidation pathway of neutrophils. The following values were obtained (n=5)

Dithranol conc µM	1	3.3	10	33	100
% of control	94	84	82	83	66
SE	4	4	1	2	6

Evidence that the platelets remained intact when incubated with dithranol was obtained by measuring the release of 14C 5HT added to the platelets prior to incubation. 5HT release was always less than 2%.

Our study shows that dithranol lowers 12(S)HETE levels. This is due to decreased production rather than enhanced enzymatic breakdown and may contribute to its antipsoriatic effects.

URINARY LEUKOTRIENE E_4 LEVELS IN SEVERE ATOPIC DERMATITIS

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Urinary leukotriene (LT) E_4 is a stable end product of LTC_4 and LTD_4 and is increased after antigen inhalation and in patients with severe acute asthma, but normal in patients with allergic rhinitis (Taylor GH, Black P, Turner N et al., Lancet 1989;i.584-587). This discrepancy may reflect the possibility that different inflammatory mediators are involved in the pathogenesis of asthma and allergic rhinitis, or that the degree of inflammation is less marked in allergic rhinitis, since a smaller surface area is involved. By studying patients with atopic dermatitis (AD), we hoped to clarify this question.

6 patients with severe AD, as defined by a twenty area severity assessment score, were studied. The patients were admitted to hospital having received no systemic medication other than antihistamines during the previous month. Antihistamines were stopped at least 72 hours prior to study and emollients only were applied to the skin during the 24 hours prior to and during urinary collection. Peak expiratory flow rate (PEFR) was measured on admission and a 24 hour urinary collection initiated from which the creatinine clearance was estimated. A 20 ml sample from each collection was spiked with 4-6000 dpm ^3H-labelled LTE_4 (as an internal standard) and stored at -80°C until assayed. Patients were then treated with topical steroids, antihistamines and emollients until the AD had cleared when the 24 hour urinary collection and PEFR were repeated. Urinary LTE_4 was measured by high-performance liquid chromatography and a radioimmunoassay.

Urinary LTE_4 levels in severe AD were within the normal range (mean ± s.d 12 55 ± 6 38 pg/µmol creatinine, normal range < 100 pg/µmol) and did not alter significantly on remission of the disease (mean ± s.d. 22.36 ± 13.86) Based on PEFR measurements, patients were not actively asthmatic. In contrast to findings in acute asthma, these results do not suggest the release of leukotrienes in vivo in patients with severe AD, a situation in which the inflammatory disorder involves a larger surface area than in allergic rhinitis but less than in asthma.

ISOTYPE OF ANTIBODIES TO INHALANT AND DIETARY PROTEINS IN CHILDHOOD ATOPIC ECZEMA

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The aim of this study was to evaluate the immunoglobulin (Ig) isotype of antibodies to house dust mite (HDM), ovalbumin (OVA) and casein (CAS) by ELISA, as well as levels of total serum Ig isotypes, in children (n=101) with atopic eczema (AE) and non-atopic age-matched controls (n=62). Total serum IgE was elevated in AE children, compared with the non-atopic controls. Mean levels of total serum IgG4 were also greatly elevated (a threefold increase) in AE patients and 33% of children had IgG4 levels >1.0g/l. Mean levels of total serum IgG2 were also significantly increased compared with controls. IgE and IgG4 levels were correlated in AE children and increased with age. In contrast, levels of total IgA1 and IgA2, determined in 25 AE children with the highest IgG4 levels, showed no significant deviations from normal and IgA2 levels were not correlated with IgG4 levels.

IgA anti-HDM and IgA anti-OVA antibodies were not detectable in AE and IgA anti-CAS antibodies were infrequent. IgG anti-OVA antibodies were restricted to the IgG4 isotype, both in AE patients and controls. Anti-CAS antibodies were also often of the IgG4 isotype, but also included IgG2 and IgG1 isotypes. In contrast, IgG anti-HDM antibodies showed no significant IgG4 antibody binding and were restricted essentially to the IgG1 isotype. Whilst one third of AE children were IgG anti-OVA, IgG anti-CAS and IgG anti-HDM antibody-positive, these antibodies were shown to be non-cross-reactive by competitive inhibitions.

Taken together, these findings can be interpreted in the context of a disorder of immunoregulation underlying AE.

THE EFFECTS OF IMMORTALISATION WITH HUMAN PAPILLOMAVIRUSES ON SECRETION OF INTERLEUKIN 6 BY ORAL KERATINOCYTES

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Chronic immunosuppression of renal transplant patients can result in development of cutaneous warts which appear to progress to squamous carcinomas in some cases. Progression is thought to be due to a defect in the host immune surveillance system allowing papillomavirus immortalised cells to become malignant. The immune system is regulated in part by small peptide regulatory molecules known as cytokines. Many of these cytokines are produced by keratinocytes themselves, including interleukins 1, 3, 6 and 8 and the colony stimulating factors. Interleukin 6 is a particularly potent activator of the host immune system through its abilities to stimulate maturation of B-cells to plasma cells and T-cell precursors to cytotoxic T-cells. With the long term aim of investigating the effects of papilloma viruses on the immune function of keratinocytes we have begun by assessing the production of IL-6 by oral keratinocytes and cell lines derived from them by transformation with papillomavirus genomes.

Oral keratinocytes were transfected with plasmids encoding the genome of HPV 16 and cloned, immortal cell lines were isolated. The production of IL-6 by parental cells and derived cell lines was assayed using the B9 hybridoma cell line which is dependent on IL-6 for its replication Supernatants from all cells contained detectable IL-6 The level of secretion from parental cells varied but was of the order of hundreds of units/ml/10^6 cells. The level of secretion from the derived cell lines was on average an order of magnitude higher Thus we conclude that papillomaviruses can disregulate IL-6 production and that this may be an important mechanism in the progression of papillomavirus immortalised cells to malignancy

C-HA RAS EXPRESSION IN NORMAL HUMAN SKIN, NON MELANOMA SKIN CANCER AND UVB-IRRADIATED SKIN.

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The ras gene family is the most frequently identified transformation-associated gene found in human solid tumours. It has been reported that the activation of c-Ha ras oncogene was observed in some cases of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) by using the DNA transfection assay or oligonucleotide mismatch hybridization In order to clarify the role of c-Ha ras expression in carcinogenesis of non-melanoma skin cancer, we studied c-Ha ras expression in 6 samples of normal human skin, 6 of UVB exposed skin 7 hrs post irradiation (Table 1) as well as in non-melanoma skin cancer (3 BCC, 1 Bowen's disease and 2 SCC) by quantitative in-situ hybridization method utilizing anti-sense RNA probe. In normal human epidermis c-Ha ras transcripts were detected thoughout the epidermis The expression of c-Ha ras in SCC was two times stronger than in normal human epidermis In Bowen's disease there was considerable variability of c-Ha ras expression However, the intensity of signals in BCC is almost the same as that of normal epidermis The intensity of c-Ha ras expression in UVB-exposed skin 7 hours after exposure was not significantly different from normal skin. Because we could not find changes of c-Ha ras expression after UVR, these results suggest that c-Ha ras oncogene expression may be involved in the late stages of malignant change in the carcinogenesis of SCC.

Table 1 C-Ha ras expression before and after UVR (mean values, grains/cell) in normal volunteer subjects

	0 MED	0.5 MED	2 MED
Basal layers	13.2 (n=6)	12.7 (n=6)	13.7 (n=5)
Malpighian layers	14.0 (n=6)	12.9 (n=6)	15.6 (n=5)
Granular layers	13.2 (n=6)	11.8 (n=6)	11.5 (n=5)

Table 2: C-Ha ras expression in normal skin and non melanoma skin cancer (mean values, grains/cell)

Normal epidermis	Granular layer	13.2 (n=6)
	Malpighian layer	14.0
	Basal layer	13.2
BCC		9.9 (n=3)
Bowen's disease	Basal layer	9.2 (n=1)
	Malpighian layer	15 8
SCC		25.4 (n=2)

IMMUNOCYTOCHEMICAL CHARACTERISATION OF HUMAN MELANOCYTES IN CULTURE.

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Melanocytes are normally recognised as dopa staining, dendritic cells. In culture however, they assume many forms, representing different stages of differentiation. We have attempted to characterise these using immunocytochemistry.

Human melanocytes were grown in TPA free medium, supplemented with bovine hypothalamic extract, 1% serum and other growth factors. They were subcultured on coverslips for dopa staining and immunocyto-chemistry using a range of antibodies including those that recognise tyrosinase (PEP-7), a tyrosinase related protein (MEL5) and premelanosomes (NKI-beteb).

In culture the melanocytes were seen as small or elongated bipolar, tripolar or dendritic cells although a few larger polygonal cells were sometimes evident. The strongest immunochemical staining was generally seen in well differentiated dendritic cells except for NKI-beteb which stained the least differentiated bipolar cells preferentially. The polygonal cells normally showed poor immunochemical staining and were dopa negative. Addition of 1mM cAMP or 1nM cholera toxin for 5 days increased dendricity and the staining intensity of all cell types with PEP-7 and MEL5. There was also an increase in staining with NKI-beteb but again this was confined mainly to the bipolar cells.

Our results confirm that human melanocytes in culture show a variety of morphological forms and demonstrate that it is possible to distinguish these different forms using immunochemical staining. This should prove useful in studying the differentiation of human melanocytes in culture.

We acknowledge support from Stiefel International.

* British Council Scholar from University of Bordeaux.

SKIN REACTIVITY TO NEUROPEPTIDES IN CHRONIC IDIOPATHIC URTICARIA (CIU)

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Neuropeptides (NP) cause mast cell degranulation and may be released in response to both physical and chemical (including histamine) stimuli An increased sensitivity to NP might explain the enhanced and prolonged skin responses to histamine observed in patients with CIU. Intradermal injections (25μl) of Calcitonin Gene Related Peptide (CGRP) 0 5-2.0μM, Substance P (SP) 0.25-1 0μM, Neurokinin A (NKN A) 0.05-0.5μM, Vasoactive Intestinal Peptide (VIP) 0.5-2.0μM, compound 48/80 1 0-10 0μg, histamine 1 0-10.0 μg and phosphate buffered saline (PBS) were administered to 10 patients with CIU, and age, sex matched controls. Flare was measured at 2, 20, 60, 120 min and wheal at 12, 30, 60, 120 min using planimetry. Cutaneous blood flow was measured at 2 and 20 min using laser Doppler flowmetry. Area under the response time curve (AUC) was calculated for each dose investigated, and the dose response for each compound analysed with three way analysis of variance

A mean increase in wheal AUC for all concentrations of VIP was observed in CIU compared to controls, $p<0.001$ (0.5μM 4986±316, 1950±108, 1 0μM: 7996±397, 4787±161, 2 0μM. 9986±484,7360±271mm^2/2h respectively, mean±SEM) Similarly, a mean increase in wheal AUC for all concentrations of histamine was observed in CIU as compared to controls, $p<0.02$ (1.0μg. 5771±152, 3932±119; 3.0μg· 7746±297, 6446±272, 10μg. 11916±494, 10650±912 mm^2/2h respectively; mean±SEM) There was no significant difference in flare or cutaneous blood flow to VIP and histamine Responses to CGRP, SP, NKN A, 48/80 and PBS were the same in urticarial and control subjects

These data may indicate an increased sensitivity of CIU microvasculature to VIP compared to controls The enhanced wheal response to histamine may also be mediated via VIP released from branches of afferent cutaneous C fibres following antidromic stimulation

THE EFFECT OF AGEING ON THE DISTRIBUTION OF AFFERENT CUTANEOUS NERVES AND NEUROPEPTIDE EXPRESSION IN HUMAN SKIN. D.W.S. Harris, [1]T. Cowen and M.H.A. Rustin, Departments of Dermatology and [1]Anatomy, The Royal Free Hospital and School of Medicine, London NW3 2QG.

Recent studies have shown substantial changes in efferent peptide-containing innervation of human skin in old age(Rahman et al J Auton Nerv Syst 1991. submitted). We have examined forearm skin of young and old healthy subjects for similar senescent changes affecting presumptive afferent nerve fibres.

Normal skin from 2 elderly (83,76yrs) and 2 young (22,24 yrs) subjects was sampled. Using standard immunohistochemical techniques cryostat sections were stained for afferent fibres using antibodies to Calcitonin gene-related peptide (CGRP) and Substance P(SP). The pan-neuronal marker PGP 9.5 was used to obtain estimates of the total nerve population. Immunofluorescent nerve fibres in the strata spinosum, germinativum and the superficial dermis were assessed visually and by image analysis.

PGP staining showed numerous fine nerve fibres penetrating from the superficial dermis into the epidermis as far as the stratum spinosum. Total nerve density was visibly reduced in the elderly. Area% of nerves in sections from elderly subjects averaged 0.4% compared to 0.8% in young. The reductions mainly affected fine varicose nerve fibres. CGRP immunoreactivity was present in skin from young patients as sparse,fine fibres with a similar distribution to PGP immunoreactive nerves, whilst SP immunoreactivity demonstrated only a few nerve fibres per section. CGRP staining was absent from superficial skin of elderly patients. CGRP immunoreactive nerves fibres constituted less than 10% of the total population of fibres as demonstrated by PGP staining, suggesting the presence of non-CGRP,non-SP immunoreactive fibres in superficial skin.

CUTANEOUS NERVE DENSITIES IN DIABETIC AUTONOMIC NEUROPATHY: A QUANTIFIED IMMUNOHISTOCHEMICAL STUDY. M.S.K. Al'Abadie, H.J. Senior, [1]M. Dent, [1]J.D. Ward and D.J. Gawkrodger, Departments of Dermatology and [1]Medicine, Royal Hallamshire Hospital, Sheffield S10 2JF

Nerve damage is a common complication of diabetes mellitus although its cause is poorly understood. The pathological process in the nerves may precede the onset of clinical changes and, with the design of a predictive test in mind, we aimed to develop a technique to quantify the nerve fibre density in the skin of diabetics with neuropathy.

Five diabetics (mean age 55 years) with peripheral and autonomic neuropathy were compared to 5 non-diabetic controls (mean age 47 years). Skin biopsies were taken from the lower leg. Standard immunoperoxidase techniques were employed on cryostat cut sections, using two anti-neurofilament monoclonal antibodies, 127.11 and UJ13A (both kindly provided by the ICRF Oncology Unit, Institute of Child Health, London). The sections were counted for area of reaction product per unit dermal area (arbitrary units) using a programmed Quantimet 970 image analyser (Cambridge Instruments).

The mean counts (in arbitrary units) for diabetics and controls were 2185 and 3321, for 127.11, and 2029 and 3163, for UJ13A, respectively. These differences were statistically significant ($p<0.01$: 2-sample t-test). These findings indicate that diabetic neuropathy is associated with a quantifiable loss of dermal nerve fibres. The predictive ability of this method in recently presenting or uncomplicated diabetics requires evaluation.

IN SITU HYBRIDISATION WITH DIGOXIGENIN LABELLED SINGLE STRANDED RNA PROBES FOR KERATIN 14 AND RARγ. Jonathan Rees & Christine Fisher, Department of Dermatology, University of Newcastle upon Tyne, UK.

Isotopic methods of performing in situ hybridisation offer superb cellular localisation, fast development times as well as increased safety. However, drawbacks of these systems to date have been the reported lack of sensitivity. We have previously shown that oligonucleotides labelled with digoxigenin are capable of working in paraffin embedded skin material including that processed by a routine histopathology laboratory. More recently we have used this technique to study keratin 14 gene expression and attempted to use this technique to look at RARγ transcripts in skin. Sections were paraformaldehyde fixed, embedded in paraffin and processed according to standard methods. Single stranded RNA probes for K14 (a gift from Elaine Fuchs, University of Chicago) and RARγ (a gift from Pierre Chambon) were hybridised to these sections in 50% formamide at 50°C. These were then washed at high stringency up to 0.1 ssc/50% formamide at 50°C. The alk phos/x-phos/NBT detection system was used as recommended by the manufacturer. Clear signal was evident with the K14 keratin probe within a few hours. Good intracellular localisation was obtained with a clear distribution of signal towards the apexes of the cell but occasionally with some nuclear staining. Within the sections it is possible to see whether a cell is undergoing mitosis or not. Results using the RARγ have been more problematic: this message is known to be at a relatively low abundance and as a low signal is apparent it is difficult to decide whether this is non-specific rather than genuine signal.

We feel this method which we have shown to work on routinely collected histopathological material offers significant advantages over radioisotopes for studying mRNAs that are relatively abundant such as keratins or other structural proteins of the skin. We are presently engaged in trying to improve the method so that it may be used to detect rarer species.

MEASUREMENT OF DISTRIBUTION AND REPAIR OF 8-METHOXYPSORALEN PHOTOADDUCTS IN HUMAN LYMPHOCYTES. A.E.M. Taylor, P. Bevilacqua, F. Gasparro, Department of Dermatology, Yale University, New Haven, CT. U.S.A.

The spontaneous DNA repair process which follows exposure to 8-methoxypsoralen (8-MOP) and UVA remains uncertain. The extent and distribution of repair of 8-MOP-DNA photoadducts have been studied. Photoadducts were produced in human lymphocytes following treatment with 10ng/ml 8-MOP and 1J/cm^2 UVA. The DNA was isolated from the cells either on Day 0 or after 2 days incubation in RPMI medium. The total number of photoadducts were determined by liquid scintillation analysis of covalently bound [^{3}H] 8-MOP. The DNA was enzymatically hydrolysed and HPLC analysis carried out. The distribution and quantification of the 4',5'-monoadducts, 3',4'-monoadducts and the crosslinks were determined, the retention times for specific photoadducts being known under the HPLC conditions employed. The total counts per minute per HPLC fraction number were measured.

On Day 0 a total of 0.4 adducts per million bases were formed from 0.17 (42%) 4',5'-monoadduct, 0.059 (15%) 3',4'-monoadduct and 0.17 (43%) crosslink. By Day 2 a total of 0.31

adducts were present, verifying 25% repair within 48 hours (Bevilaqua PM, Edelson RL, Gasparro FP. HPLC analysis of 8-MOP monoadducts and crosslinks in lymphocytes and keratinoocytes. J Invest Dermatol 1991; 97: In press). The 4',5' adduct was reduced by 18% to 0.14 per million bases, the 3',4' adduct by 39% to 0.036 per million bases and the crosslink by 29% to 0.12. These data indicate the preferential repair of the 3',4'-monoadducts and over the 4',5'-monoadduct in the repair process.

QUANTIFICATION OF CELLULAR PROLIFERATION IN ACNE USING THE MONOCLONAL ANTIBODY Ki-67.

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Acne is characterised by hypercornification of the pilosebaceous duct, but the mechanism by which hypercornification occurs is uncertain. We investigated proliferation in normal and acne follicles and in the interfollicular epidermis using the monoclonal antibody Ki-67 which reacts with a nuclear antigen expressed by cells in the late G1,S,M and G2 phases of the cell cycle.

Cryostat sections (6um) of biopsies from the interscapular region from acne patients and normal volunteers were stained with Ki-67 antibody and counterstained with methyl green. The number of Ki-67 positive nuclei were counted and expressed as a percentage of the total number of nuclei in the ductal epithelium. In addition, at least three regions of epidermis were counted per section: two areas remote from the follicle and one perifollicular area.

In normal follicles ($n=8$), 12.7% ± 1.94% of the cells were Ki-67 positive. This value rose significantly to 21.9% ± 2.57% ($p<0.05$) for non-inflamed lesions ($n=8$). It was impossible to count the nuclei of follicular epithelium of inflamed lesions because little of it remained intact. The distribution of the Ki-67 positive cells was uneven in both healthy and diseased follicles and was not restricted to either the acroinfundibulum or infrainfundibulum.

In normal interfollicular epidermis, Ki-67 positive cells represented 6.3% ± 1.3% ($n=6$) of the total. This value was not significantly different from the value obtained for interfollicular epidermis near non-inflamed lesions (10.5% ± 2.6% ($n=7$)). However, the number of Ki-67 positive nuclei in the interfollicular epidermis near inflamed lesions was significantly higher than either of these two values: 25.2% ± 3.6% ($n=5$) $p<0.05$. In inflamed skin considerable numbers of suprabasal positive nuclei were observed.

Our results with Ki-67 confirm those derived using tritated thymidine incorporation. Scoring Ki-67 positive cells thus appears to offer a more convenient method than tritiated thymidine incorporation for quantifying keratinocyte proliferation. The results confirm that ductal hyperproliferation occurs in acne. Our data also supports the unifying concept of dermal-epidermal interdependence as it is likely that epidermal hyperproliferation is mediated by the release of inflammatory cytokines from the dermis.

SELECTIVE INVOLVEMENT OF KERATINS K1 AND K10 IN THE CYTOSKELETAL ABNORMALITY OF EPIDERMOLYTIC HYPERKERATOSIS

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Aggregation of keratin filaments within spinous and granular epidermal keratinocytes is a characteristic feature of Epidermolytic Hyperkeratosis as seen in Bullous Congenital Ichthyosiform Erythroderma (BCIE) and other skin conditions. The histological distribution and keratin expression of the altered keratin filaments was investigated to determine whether the aggregation was specific to any particular keratin(s) Skin samples from four patients (three with BCIE and one with Epidermal Nevus type of Epidermolytic Hyperkeratosis) were subjected to electron microscopy and immunoelectron microscopy, using monospecific antibodies against the four major epidermal keratins K1, K5, K10, and K14. Conjunctival samples and confluent culture of epidermal keratinocytes from one patient with BCIE were also examined by electron microscopy. Electron microscopically distinct keratin filament aggregates were distributed suprabasally in the epidermis, the infundibular outer hair root sheath, the sebaceous ducts and the sweat ducts, following the distribution pattern of K1 and K10. The abnormal keratin filaments were not found in conjunctival epithelium or in cultured keratinocytes, where K1 and K10 are not or are only minimally expressed. Immunoelectron microscopy confirmed that K1 and K10 were expressed in the suprabasal keratin filaments including aggregated ones. Whereas K5 and K14 were strongly expressed in the non-aggregated filaments of basal and suprabasal keratinocytes, labelling for these keratins was weak or absent within the K1/K10 positive aggregates. These results suggest that the keratin filament abnormality may principally involve K1 and K10 and that Epidermolytic Hyperkeratosis might be a disorder of one or both of these keratins

EXPRESSION OF VASCULAR CELL ADHESION MOLECULE-1 (VCAM-1) IN NORMAL AND INFLAMED SKIN.

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Vascular cell adhesion molecule-1 (VCAM-1) is a novel endothelial adhesion protein with adhesive properties for lymphocytes. It is regulated in endothelial cell culture by interleukin-1 and tumour necrosis factor α, both of which are potentially keratinocyte derived, but there is at present little information on its expression in human skin.

Skin biopsies were obtained from patients with allergic contact dermatitis ($n=5$, $t=48$-96hr), chronic plaque psoriasis ($n=5$) and normal skin ($n=5$) 5μm frozen sections were examined using a monoclonal antibody to VCAM-1 in a three layer immunoperoxidase reaction. In normal skin, VCAM-1 was expressed by dendritic cells in the perivascular region but not by endothelial cells. Also, occasional interstitial dermal dendritic cells were positive but keratinocytes did not express VCAM-1. In acute contact dermatitis, there was marked VCAM-1 upregulation with prominent endothelial cell and dermal dendritic cell positivity. In addition, a small component of the infiltrating inflammatory cell population expressed VCAM-1. In chronic psoriasis, VCAM-1 was present on perivascular and interstitial dermal dendritic cells but upper dermal capillaries, which on serial sectioning expressed ICAM-1 and ELAM-1, did not express VCAM-1.

These findings demonstrate a low level of constitutive VCAM-1 expression in normal skin which may be differentially regulated in acute and chronic cutaneous inflammation. Not only is VCAM-1 expressed by endothelium and inflammatory cells, but also by perivascular and interstitial dermal cells of dendritic morphology: this molecule may be of importance in trafficking of lymphocytes into and within inflamed skin.

HLA & INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) EXPRESSION IN ALOPECIA AREATA (AA) IN-VIVO AND IN-VITRO: THE ROLE OF CYTOKINES.

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Aberrant HLA class II expression in hair follicle epithelium has been proposed as a possible primary abnormality in AA but recent work favours induction by cytokine release from infiltrating lymphocytes as the cause of this. ICAM-1 expression is crucial to lymphocyte trafficking in inflammatory dermatoses and usually precedes expression of HLA DR. In this study, patterns of HLA and ICAM-1 expression in tissue sections from 9 patients with alopecia areata and 2 normal controls were compared with patterns induced in-vitro by interferon-gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and granulocyte-macrophage colony stimulating factor (GM-CSF) in follicles from 6 normal subjects and non-lesional scalp from 4 AA patients maintained in short-term organ culture for 2 to 7 days.

In tissue sections from AA, there was close spatial correlation of ICAM-1 with HLA-DR; prominent staining being noted in the dermal papillae and precortical matrix of lesional anagen follicles. In cultured follicles, dose-dependent induction of HLA I, DR and ICAM-1 by IFN-γ, and HLA I and ICAM-1 but not HLA DR by TNF-α were observed in follicular epithelium, predominantly in the outer root sheath. Both IFN-γ and TNF-α induced ICAM-1 and HLA I but not HLA DR in the dermal papilla. However, we found no evidence of altered sensitivity to induction of HLA by IFN-γ, TNF-α or GM-CSF in AA.

The patterns of HLA and ICAM-1 expression induced in hair follicles in-vitro were closely similar to those reported in epidermal keratinocytes but the staining distribution was quite different from that seen in-vivo in AA. These findings suggest that local cytokine release is not the sole determinant of aberrant HLA expression in AA.

ANDROGEN RECEPTOR LOCALIZATION IN HUMAN SKIN AND PILOSEBACEOUS UNITS: EVIDENCE THAT CELLS OF DERMAL PAPILLA ARE THE TARGETS OF ANDROGEN ACTION ON HAIR GROWTH.

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To understand the mechanisms by which androgens regulate hair growth and sebum production it is essential to identify potential target cells for androgen action in skin. A mouse monoclonal antibody against the N-terminal region of human androgen receptor (AR) was used to localize receptor in frozen serial sections of male and female skin from various body sites (scalp, face, limb & genitalia) by an indirect immunoperoxidase technique. AR staining was seen in cell nuclei in dermal papilla cells of a proportion of hair follicles in anagen and telogen from genital, beard, temporal and scalp regions. The frequency of receptor staining was lowest in female scalp dermal papillae. AR staining was not seen in germinative matrix, root sheaths or shaft of the hair. In 21/21 specimens, specific nuclear staining was seen in basal and differentiating sebocytes. In some pilosebaceous ducts basal and suprabasal keratinocytes stained intensely. AR was also seen in epithelial cells of apocrine and eccrine sweat glands and in dermal fibroblasts of the genital skin. It appears that dermal papilla cells are the site of androgen receptor expression in hair follicles. This supports the notion that androgens modulate cellular interactions between dermal papilla and germinative matrix. In contrast, androgens may regulate sebum production by acting directly on basal and differentiating sebocytes. The expression of AR in infundibular keratinocytes suggests that androgens may influence pilosebaceous duct keratinization.

MINOXIDIL OVER-SULPHATES GLYCOSAMINOGLYCANS IN EXTRACELLULAR MATRIX OF CULTURED HUMAN HAIR PAPILLA CELLS.

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The presence of glycosaminoglycans (GAG) in the hair follicle dermal papilla (DP) varies during the hair growth cycle suggesting that GAG may have a role in controlling hair growth. We have also found that cells cultured from human DP continue to lay down a GAG-rich extracellular matrix (ECM) in vitro. In this study we have examined the effect of minoxidil (Mx) on GAG synthesis by cultured human DP cells.

7 DP cell lines were grown to confluence in 24-well plates and then incubated with Mx (0.02-40ug/ml) for 48h. ^{35}S-sulphate (3MBq/ml) and ^{3}H-glucosamine (0.4MBq/ml) were added to each well for the last 24h. Radiolabel incorporation into ECM GAG was measured by scintillation counting. The results were expressed as dpm/ug cell protein.

Mx stimulated mean ^{35}S-sulphate incorporation into ECM GAG by 10% (range 0-39%) at 0.02, 0.2 and 2ug/ml but had no effect on ^{3}H-glucosamine. The stimulatory effect was seen in 4 out of 7 cell lines.

These results suggest that Mx increases the level of sulphation of DP GAG at pharmacologically relevant concentrations but has no effect on total GAG synthesis. Increased sulphation of GAG, which raises their negative charge and hydrophilic nature, may increase DP volume and hair diameter. The possibility that Mx is over-sulphating a specific GAG is currently being investigated by enzyme/chemical depolymerisation and HPLC analysis of disaccharides.

HUMAN MELANOMA CELL-DERIVED FACTOR(S) STIMULATE FIBROBLAST HYALURONATE SYNTHESIS.

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It has become increasingly apparent that some form of desmoplasia occurs surrounding many growing, invading and metastasising tumours, and that this change in tissue architecture may facilitate tumour invasion. These tumour-induced changes are, in many instances, similar to those observed in healing wounds, and are characterised by an increased production of hyaluronate, suggesting that a hyaluronate-rich matrix favours cell migration/invasion. Here we present evidence that melanomas may express factors that stimulate fibroblast-mediated matrix changes which facilitate tumour invasion.

Human B0008 melanoma cells were grown in Eagle's MEM containing 10% foetal calf serum for 3 days, after which the medium was changed, and the cells incubated for a further 2 days. This tumour-conditioned medium, or fibroblast-conditioned medium (control) was added to subconfluent adult scalp fibroblasts which were then incubated for 2 days in the presence of ^{3}H-glucosamine (5µCi/ml) and $^{35}SO_4$ (20µCi/ml). Glycosaminoglycans (GAGS) were isolated by ion-exchange chromatography and identified by their susceptibility to degradation by specific enzymes.

Human melanoma cell-conditioned medium stimulated a 9.2 fold increase in fibroblast hyaluronate synthesis, and also increased its molecular weight substantially. ^{3}H-glucosamine incorporation into sulphated GAGS was increased 2.8 fold, but sulphate incorporation increased only 1.06 fold, suggesting that their degree of sulphation was significantly reduced. The proportions of heparan sulphate and chondroitin sulphate remained unaltered, while the tumour cell-conditioned medium-treated fibroblasts appeared more elongated.

This dramatic increase in both the synthesis and molecular weight of hyaluronate, following treatment of fibroblasts with melanoma cell-conditioned medium, suggests that the matrix changes observed surrounding invading tumours in vivo may be mediated by normal fibroblasts, induced to do so by tumour-derived factors.

INTRACELLULAR EXPRESSION OF TYPE VII COLLAGEN DURING WOUND HEALING IN SEVERE RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA AND NORMAL HUMAN SKIN.

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The severe form of recessive dystrophic epidermolysis bullosa (SRDEB) is characterized morphologically by an absence of recognizable anchoring fibrils at the dermo-epidermal junction and blister formation below the lamina densa. Anchoring fibrils are mainly composed of type VII collagen (TVIIC) and an absence of immunofluorescence staining with anti-TVIIC antibodies has been widely used in the diagnosis of SRDEB. It is not clear, however, whether the abnormality of TVIIC in SRDEB represents complete absence, altered synthesis, aberrant assembly or excessive breakdown of the TVIIC molecule. Immunofluorescent studies using the LH 7 2 monoclonal antibody (recognizing the globular region of TVIIC) were performed on biopsies of intact skin and healing skin 7-14 days after dermatome injury in five patients with SRDEB and in five normal human skin controls. Baseline immunofluorescence showed completely absent staining of the epidermis, dermis and dermo-epidermal junction in SRDEB samples and bright linear dermo-epidermal junction fluorescence in normal human skin. In all the 7-14 day post-wounding samples in normal human skin, TVIIC expression was noted in basal cells as well as in a continuous or interrupted linear distribution at the basement membrane zone. In all the corresponding SRDEB samples, LH 7:2 fluorescence was also seen with varying intensity within basal and lowermost suprabasal cells and occasionally at the dermo-epidermal junction. Low levels of intracellular type IV collagen were seen in both groups but only in those samples taken 7-9 days after injury; later biopsies showed merely continuous dermo-epidermal junction staining. No skin specimens had intracellular anti-laminin fluorescence. The finding of anti-TVIIC immunofluorescent staining within the lower epidermis in SRDEB during wound healing implies that these keratinocytes have an inherent capacity to synthesize TVIIC, or at least the non-collagenous globular region of TVIIC. This suggests that under certain conditions, SRDEB epidermis might be able to synthesize a component of anchoring fibrils. Further work with molecular markers should help to determine whether the TVIIC abnormality is at the translational or post-translational level.

TENASCIN EXPRESSION IS INDUCED DURING ACNE VULGARIS.

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Tenascin is an extracellular matrix glycoprotein, which may be involved in cellular proliferation and migration and may function as a growth promoter. The distribution of tenascin suggests it is important in the embryonic development and regeneration of hair follicles. Tenascin has a restricted distribution in normal skin but is induced in wound healing and hyperproliferative diseases. Since acne is accompanied by considerable follicular damage and the remodelling processes are poorly understood, we investigated the expression of tenascin in acne.

Biopsies obtained from patients with (n=15) and without acne (n=5), were cryostat sectioned and stained with tenascin antiserum (a kind gift from Dr. Eleanor Mackie, Dept. Forschung, Kantonsspital CH-4031, Basel, Switzerland). The results confirm the previously reported restricted and patchy distribution of tenascin beneath the dermo-epidermal junction in skin and around the blood vessels. We demonstrated tenascin expression as a continuous band around the normal pilosebaceous duct with very limited expression around the sebaceous gland. In early non-inflamed lesions there was no increase in tenascin distribution. In inflamed lesions however, tenascin was diffusely distributed throughout the perifollicular dermis. The area involved being considerably greater (by 100 fold, $p<0.001$) when compared with normal follicles and non-inflamed lesions. This increase was particularly evident in lesions in which there was duct rupture and lymphocytic infiltration. Thus, increased tenascin expression appears to be associated with the development of inflamed lesions in acne. However, it is not known whether the induction of tenascin precedes inflammation or is a consequence of the inflammation and the wound healing process.

A RE-EVALUATION OF THE USE OF HALF-FACE STUDIES TO SCREEN NEW ACNE TREATMENTS.

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Half-face studies to compare products such as topical antibiotics have had a poor reputation for yielding unreliable results due to suspected treatment translocation (Marples RM, Kligman AM *Br J Dermatol* 1973; 88:61-67). However, such studies could be advantageous as they reduce the number of people required for the screening of novel acne treatments. This could be beneficial in both cost and time factors.

We have re-evaluated the situation and attempted to determine whether there is significant treatment translocation. We performed 5 studies, with benzoyl peroxide (BP) acting as the positive control each time. On the opposing side of the face, placebo or 4 other novel compounds were used. In each study we investigated 10-13 patients at 4 and 8 weeks after start of treatment. All acne lesions were counted, skin surface microflora were quantified and by using an organic solvent wash, samples were taken from the face in which the presence of BP could be analysed by HPLC.

The BP treated side always showed a significant decrease in (a) non-inflamed/inflamed lesions and (b) propionibacteria (except in 1 case) at 4 and 8 weeks. Reproducability of the BP therapy was confirmed using the K-Wallis test (no significant differences seen).

BP was present significantly more often on the BP treated side compared to the contralateral side ($p<0.05$). Furthermore, a significant reduction in propionibacteria was apparent when comparing the BP and contralaterally treated sides ($p<0.01$). No test compound was as effective in reducing both the number of lesions and target micro-organisms as BP despite 3 of them having some action against P. acnes *in vitro*.

We conclude that the technique can distinguish between 2 different compounds and that very little therapy translocation has occurred. This requires further investigation but our data supports the potential benefit of half-face studies.

IMMUNOFLUORESCENCE STUDIES IN CICATRICIAL PEMPHIGOID

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We studied various tissues and sera from 34 patients with cicatricial pemphigoid (CP) using direct and indirect immunofluorescence techniques.

Biopsies from clinically uninvolved bulbar conjunctiva, oral mucosa and perilesional skin were examined for the presence of *in vivo* bound immunoreactants. A linear deposition of IgG and C3 alone or in combination were found in 44% conjunctiva, 37 5% skin and 35.5% oral mucosal biopsies. IgA and IgM were rarely found. Overall 41% of biopsy specimens examined had positive direct immunofluorescence (DIF) but this was not related to the distribution of clinical lesions.

Indirect immunofluorescence (IMF) testing was performed using skin and mucous membranes, i.e. vaginal mucosa, oral mucosa, human conjunctiva and pig's bladder to determine which substrate was the most sensitive in detecting circulating antibodies in CP patients. All the substrates except pig's bladder were used both intact and split chemically through the lamina lucida by incubation with IM NaCl. The use of human mucosal substrates failed to increase the sensitivity of IMF testing. Split human skin was the best substrate and superior to split mucous membranes and this was site dependent. 26% of patients had detectable circulating IgG antibodies and sera from 6% of patients bound C3c when intact skin was used.However, with split skin, IgG antibodies were detected in 35% of patients, IgA antibodies in 18% of patients, one patient had circulating IgM antibodies and 15% of patients bound C3c.

The sera were usually positive at a higher titre on split skin compared with intact skin, but the titres did not correlate with extent or activity of disease. A variation in titre was observed in 29% of patients with time. All the positive sera except one produced linear fluorescence on the epidermal side of the split indistinguishable from the staining pattern observed in bullous pemphigoid (BP)

The immunopathological findings in CP are similar to BP. The pattern of antibody fluorescence on the roof of the split suggests that the CP target antigen is epidermally derived. Our results do not indicate a specificity of the CP antibody for antigenic material in mucosal tissues. By using sensitive assays like chemically split skin for IMF testing, antibody detection in CP was considerably increased, and in particular, IgA antibodies were detected.

This study was funded by an Oxfordshire Health Authority Research Grant

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THE ROLE OF *MALASSEZIA FURFUR* SEROVARS A, B AND C IN PITYRIASIS VERSICOLOR AND SEBORRHOEIC DERMATITIS

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Malassezia furfur has been implicated as the aetiological agent of several dermatoses, including pityriasis veriscolor and seborrhoeic dermatitis Recent studies have differentiated 3 serovars of *M furfur*, designated A, B and C (Cunningham *et al*, J Appl Bacteriol, 1990, 68, 439) The aim of this study was to determine whether any serovar or combination of serovars was associated with pityriasis versicolor and/or seborrhoeic dermatitis by measuring the carriage of *M furfur* serovars A, B and C and cell mediated immune responses to *M furfur* A, B and C in patients and controls

The population densities of *M furfur* A, B and C, *Propionibacteria* and *Micrococcaceae*, were determined on the forehead, both cheeks, chest and back of 10 patients with pityriasis versicolor, 10 patients with seborrhoeic dermatitis and 10 normal controls, using the Williamson and Kligman Scrub technique *M furfur* isolates were differentiated into serovars A, B and C by indirect immunofluorescence using a panel of anti-*M furfur* rabbit sera. Cell mediated immunity to the 3 serovars was determined using lymphocyte transformation (LT) and leucocyte migration inhibition (LMI) assays

All results were analysed by ANOVA and population densities of organisms expressed as mean log colony forming units per cm^2 skin ± 1/2 minimum significant difference (MSD). The population densities of both *Micrococcaceae* and *Propionibacteria* varied significantly between groups For *Micrococcaceae* the highest densities (2 94 - 4 85 ± 0 45) were found on seborrhoeic dermatitis patients, whilst the highest densities of *Propionibacteria* (4 65 - 5 72 ± 0 8) were found on controls

As found previously *M furfur* serovar A predominated on the chest and back of controls (2 83 - 3 50 ± 0 47) and this was also the case in both patient groups On the forehead of controls, serovar C was present in significantly higher numbers (2 07 ± 0 47) than A or B (0 81 - 0 82 ± 0.47) In both patient groups however the densities of serovar C present were lower and, hence, there was no significant difference in the densities of serovars For the seborrhoeic dermatitis patients the lesional and non-lesional sites were compared On involved forehead sites and on controls serovar C was present in significantly higher numbers (2.07 - 2 39 ± 0 47) than A or B (0 26 - 0 82 ± 0.47) On uninvolved forehead sites the density of serovar C was at a similar level to the other serovars There were no significant differences between LT responses to A, B or C in patients or control groups. In the LMI however pityriasis versicolor patients showed a greater response to serovar B (Migration Index ±1/2 MSD = 0 43 ± 0 22) than controls (1 07 ± 0 22) This was not the case in seborrhoeic dermatitis patients where the response to all 3 serovars was similar There was no significant difference in the responses to A (0 83 - 1.15 ± 0 22) or C (0 65 - 1 06 ± 0 22) between patients and controls

Thus, this data failed to provide evidence for the association of any serovar with seborrhoeic dermatitis or pityriasis versicolor The results obtained contrast with previously published work which found a decreased cell mediated immune response to *M furfur* in patients with pityriasis veriscolor

British Journal of Dermatology (1991) 125, 496–499.

Correspondence

Hereditary epidermolytic palmoplantar keratoderma with knuckle pad-like lesions over the finger joints

SIR, Hereditary epidermolytic palmoplantar keratoderma (Vörner type) is a rare genodermatosis, characterized histologically by epidermolytic hyperkeratosis. Since it was first described in 1901,[1] only a few cases with involvement of the dorsal aspects of the hands have been reported.[2,3] We report a case of this disease in which there were knuckle pad-like lesions over the dorsal aspect of the finger joints.

A 32-year-old Japanese man attended our clinic for the treatment of palmo-plantar cutaneous lesions, which had been present from the age of 3 or 4 months. On examination the patient had a sharply defined palmoplantar keratoderma. Knuckle pad-like hyperkeratotic plaques were present on the dorsal aspect of all the proximal interphalangeal joints of the fingers except the left thumb (Fig. 1). There were no other cutaneous involvements and there were no abnormalities of the hair, nails, teeth or of the mucous membranes. The patient was otherwise healthy. The cutaneous lesions were asymptomatic and there was no hyperhidrosis. His father had had the same skin disease.

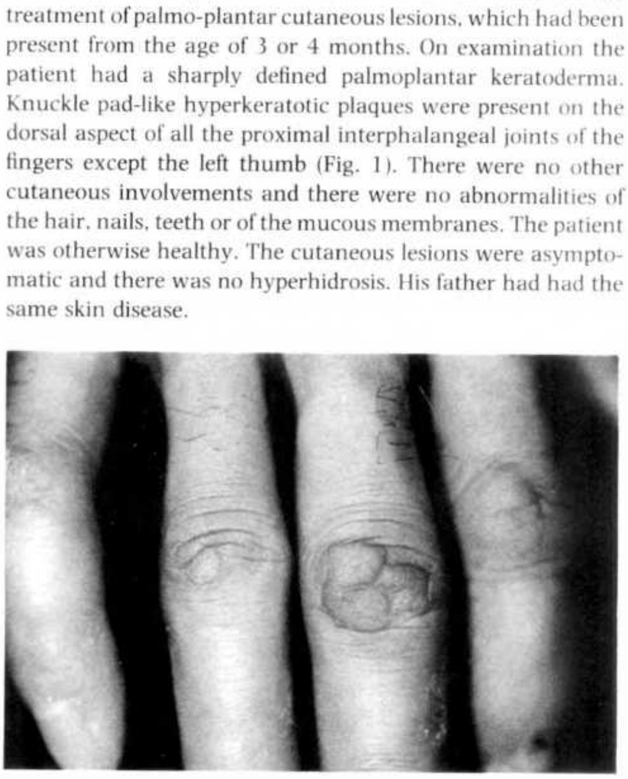

Figure 1. Knuckle-pad-like lesions over the dorsal aspect of finger joints.

Biopsies taken from the left palm and from a knuckle-pad-like lesion on the left second finger showed a compact orthohyperkeratosis, hypergranulosis and acanthosis. The granular layer and the upper third of the Malpighian layer contained basophilic granular material that varied in size and the underlying dermis showed no significant changes (Fig. 2). The histological findings were compatible with the diagnosis of an epidermolytic hyperkeratosis. Electron microscopy revealed deposits of large keratohyalin granules with clumped tonofibrils in the cells of the middle spinous layer and large, coarse masses of keratohyalin granules were present from the upper spinous to the granular layers. Immunohistochemical studies using a panel of antikeratin monoclonal antibodies showed positive reactions for 68, 58, and 56·5-kDa proteins as seen in normal epidermal cells.

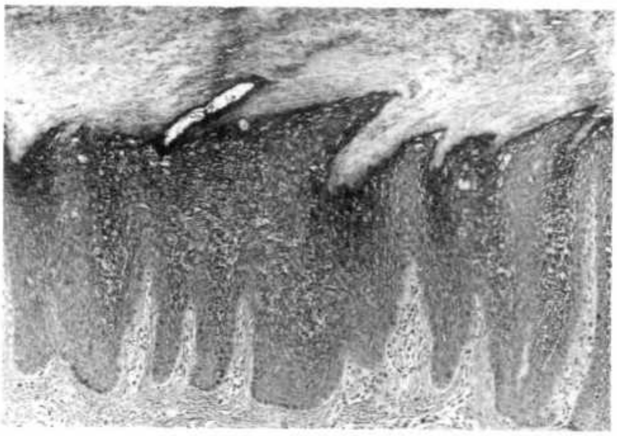

Figure 2. Biopsy of knuckle-pad-like lesion showing epidermolytic hyperplasia (haematoxylin and eosin, × 125).

Hereditary epidermolytic palmoplantar keratoderma is a rare, autosomal dominant disease that belongs to the group of hereditary palmoplantar keratodermas. There have only been 15 reports of this condition in the Japanese literature since 1964, and even in the English literature only 22 reports since 1901. The disease involves the entire surface of palms and soles while in rare cases, small hyperkeratotic plaques showing knuckle-pad-like lesions are present on the dorsum of the hands. In our case, the knuckle-pad-like lesions had been present over the dorsal aspect of each finger joint except the thumb since he was 9 years old.

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References

1 Vörner H. Zur Kenntnis des Keratoma hereditarium palmare et plantare. *Arch Dermatol Syph (Berlin)* 1901; **56**: 3–31.

2 Blasik LG, Dimond RL, Baughman RD. Hereditary epidermolytic palmoplantar keratoderma. *Arch Dermatol* 1981; **117**: 229–31.

3 Inoue Y, Yoshida J. Hereditary epidermolytic palmoplantar keratoderma. *Hifubyoh-shinryoh* 1981; **3**: 125–8 (in Japanese).

Generalized hair follicle hamartoma with a positive anti-acetylcholine receptor antibody

SIR, We would like to report the rare condition of generalized hair follicle hamartoma in association with alopecia and a positive anti-acetylcholine receptor antibody. This association has been reported only twice before.

A 33-year-old Vietnamese woman presented with induration of the glabella, eyebrows and nasolabial folds with loss of eyebrow hair over the previous year. She was well and had no ptosis, diplopia, fatigue on upward gaze or weakness suggestive of myasthenia gravis. On examination there were erythematous, indurated plaques involving the glabella and eyebrows with loss of hair from the eyebrows and lower eyelashes. There were indurated papular plaques in the nasolabial folds (Fig. 1) and a patchy alopecia of the scalp with no associated iflammation or scarring. The trunk and limb hair was absent. he refused to allow an examination of the pubic and axillary air but said that it was normal.

Histology of a biopsy from the glabella showed cords and islands of basaloid epithelium in a cellular stroma with peripheral pallisading and keratin cyst formation. The intradermal cords of proliferating epithelium were predominantly orientated at right angles to the surface. No sebaceous gland differentiation was apparent in the tumour (Fig. 2). There were structures similar to vellus hairs and in some areas the keratin filled cysts had undergone dystrophic calcification. In any small area the features were indistinguishable from a trichoepithelioma, but the vertical orientation of the epithelial structures is a feature of the rare condition of generalized hair follicle hamartoma. Immunocytochemistry was performed on formalin-fixed tissue with a panel of seven antikeratin antibodies (AE3, AE2, AE1, PKK1, Leu 5, LP34, CAM 5·2) and there was no positive labelling. A serum anti-acetylcholine receptor antibody was present in a titre of 1·2 nmol/l. The full blood picture was normal and the ESR was 16 mm/h. The RAHA was negative and the antinuclear antibody 1 : 10. The remainder of the autoantibody screen including striated muscle antibody was negative. The tissue typing was HLA A11, W33/BW58, W60/CW3, W7/BW4, W6.

Two previously reported cases of generalized hair follicle

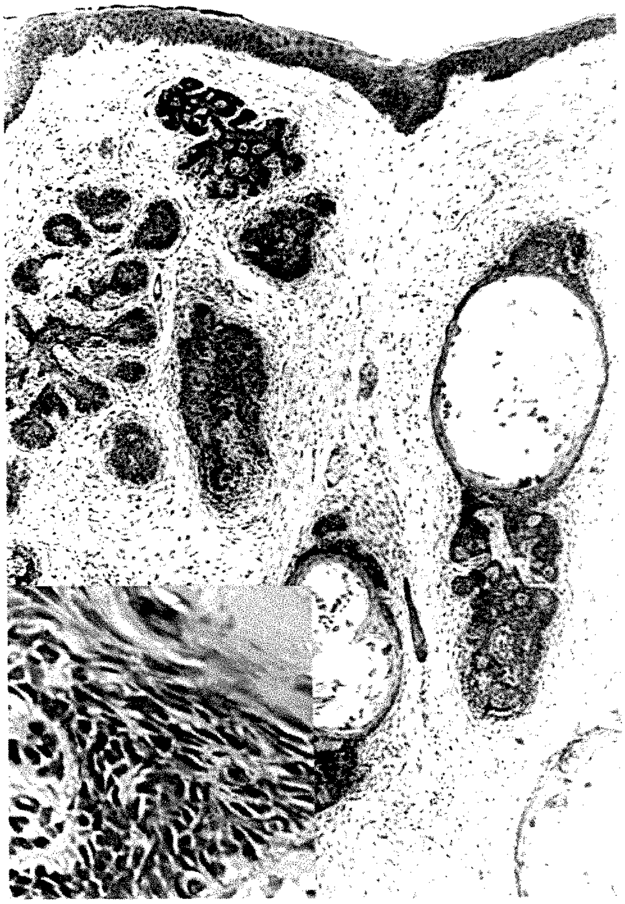

Figure 2. Intradermal cords of proliferating basaloid cells associated with keratin cysts (×40). Inset: basaloid cells with peripheral pallisading and abrupt change into parakeratin.

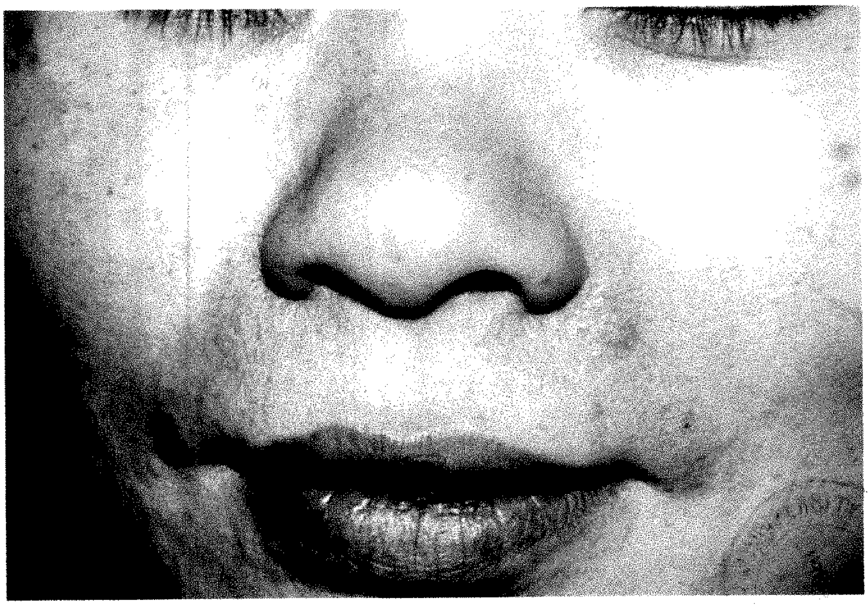

Figure 1. Indurated papular plaques in nasolabial folds.

hamartoma were associated with pre-existing myasthenia gravis.[1,2] Our patient has no clinical evidence of myasthenia gravis but had a positive anti-acetylcholine receptor antibody. This diagnostic test is highly specific, and positive in 88% of patients with the clinical features of myasthenia gravis. Biological false positives are rare, but can occur in first degree relatives of myasthenia patients, or in the elderly with a predisposition to autoimmunity. A positive titre therefore suggests that our patient may have subclinical disease and may later develop myasthenia.[3] The association of HLA A1, B8 and/DR3 with young onset myasthenia gravis and HLA A3 B7 DR2 with older onset disease is well recognized in Caucasians.[4] However, studies of Chinese patients in Hong Kong[5] and amongst the Japanese,[6] have shown distinct differences in the patterns of disease and HLA associations. In the Chinese the haplotype HLA A2, BW46, DR9 appears to be an analogue of the Caucasian haplotype A1, B8, DR3 and predisposes to similar diseases.[7] Our patient did not have these HLA-A or B antigens. DR typing was not performed and she has refused to have any further investigations.

A similar patient with facial papules, alopecia and myasthenia gravis has been reported[8] but this case was diagnosed as having generalized trichoepithelioma on the basis of focal alkaline phosphatase activity and positive labelling with basal keratinocyte markers on frozen tissue sections. We did not have the opportunity to perform similar studies but immunostaining for keratins on formalin-fixed tissue from our patient was negative. We have classified this case as a generalized hair follicle hamartoma on clinical and histological criteria[1,2] and report the case to increase awareness of this rare syndrome and to alert dermatologists to the association of alopecia and myasthenia gravis.

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References

1 Brown AC, Crounse RG, Winkelmann RK. Generalised hair follicle hamartoma associated with alopecia, aminoaciduria and myasthenia gravis. *Arch Dermatol* 1969; **99**: 478–93.
2 Ridley CM, Smith N. Generalised hair follicle hamartoma associated with alopecia and myasthenia gravis: report of a second case. *Clin Exp Dermatol* 1981; **6**: 283–9.
3 Vincent A, Newsom-Davis J. Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. *J Neurol Neurosurg and Psych* 1985; **48**: 1246–52.
4 Compston DAS, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain* 1980; **103**: 579–601.
5 Hawkins BR, Yu YL, Wong V *et al.* Possible evidence for a variant of myasthenia gravis based on HLA and acetylcholine receptor antibody in Chinese patients. *Quart J Med* 1989; New series 70, **263**: 235–41.
6 Kida K, Hayashi M, Yamada I *et al.* Heterogeneity in myasthenia gravis: HLA phenotypes and autoantibody responses in occular and generalised types. *Ann Neurol* 1987; **21**: 274–8.
7 Hawkins BR, Serjeantson SW, Higgins DA. Distribution and co-occurrence of MHC Class I, II and III markers in Southern Chinese: implications for autoimmune disease. *Dis Markers* 1988; **6**: 237–45.
8 Starink T, Birgette Lane E, Meyer C. Generalised trichoepitheliomas with alopecia and myasthenia gravis: clinicopathologic and immunohistochemical study and comparison with classic and desmoplastic trichoepithelioma. *J Am Acad Dermatol* 1986; **15**: 1104–12.

Chronic cutaneous graft-versus-host disease: successful treatment with UVB

SIR, Graft-versus-host disease (GVHD) is a major complication of allogeneic bone-marrow transplantation, resulting in a considerable morbidity and mortality.[1] Chronic GVHD starts 3 or more months after transplantation in about 25% of patients and is frequently preceded by an acute reaction.[2] Cutaneous symptoms start with erythematous papules and macules that are followed in most patients by scaling and hyperpigmentation. The skin eventually resembles that seen in systemic sclerosis with poikiloderma, ulceration, sclerosis and contractures particularly of the extremities.[1,2] The clinical response to systemic immunosuppressive therapy with corticosteroids, cyclosporin A, methotrexate, azathioprine and cyclophosphamide is often unsatisfactory and these treatment modalities are often limited by toxic side-effects.[2] A beneficial effect of PUVA therapy on the cutaneous signs and symptoms of GVHD has been reported[3–5] and this has been attributed to the immunosuppressive or immunomodulating effect of the treatment.

A 45-year-old man with chronic GVHD was referred to our department because of a poor response to prednisone therapy, this being 20 mg per day. There was generalized erythema, papules, scaling and hyperpigmentation of the skin, and he had intense pruritus. Treatment with PUVA was considered not to be appropriate because of severe liver dysfunction, and although clinical experience with UVB treatment for GVHD was limited[6,7] we decided to give him a course of treatment. He had treatment with UVB three times a week for 3 weeks, with a total dosage of 0·6 J/cm², and this resulted in a marked improvement to his skin and the itching disappeared completely. He had a relapse of symptoms 3 weeks after discontinuing therapy with UVB and was given a second course three times a week, receiving a total dosage of 4 J/cm² of UVB. It was noted that during this period there was an increase in erythema and scaling of skin, probably as a consequence of the higher dosage of UVB. These symptoms disappeared after completing treatment and so far no further treatment has been necessary. The dosage of oral prednisone throughout treatment with UVB remained unchanged at 20 mg daily.

We suggest that low-dosage UVB therapy can be a successful additional therapy in the management of chronic GVHD, especially if PUVA treatment is not well tolerated or is contraindicated.

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References

1 Matsuoka, LY. Graft versus host disease. *J Am Acad Dermatol* 1981; 5: 595–9
2 Shulman HM, Sullivan KM, Weiden PL *et al.* Chronic graft-versus-host syndrome in man: a long-term clinico-pathologic study of 20 Seattle patients *Am J Med* 1980, 69: 204–17.
3 Hymes SR, Morison WL, Farmer ER *et al.* Methoxsalen and ultraviolet A radiation in treatment of chronic cutaneous graft-versus-host reaction *J Am Acad Dermatol* 1985, 12: 30–7.
4 Atkinson K, Weller P, Ryman W, Biggs J PUVA therapy for drug-resistant graft-versus-host disease. *Bone Marrow Transplant* 1986; 1: 227–36.
5 Volc-Platzer B, Honigsmann H, Hinterberger W, Wolff K Photochemotherapy improves chronic cutaneous graft-versus-host disease. *J Am Acad Dermatol* 1990, 23: 220–8.
6 Torinuki W, Mauduit G, Guyotat D *et al* Effect of UVB radiation on the skin after allogeneic bone marrow transplantation in man *Arch Dermatol Res* 1987; 279: 424–6.
7 Glazier A, Morison WL, Bucana C *et al* Suppression of epidermal graft-versus-host disease with ultraviolet radiation. *Transplantation* 1984; 37: 211–3.

Clearing of pyoderma gangrenosum by intralesional cyclosporin A

SIR, Pyoderma gangrenosum (PG) is a rare, inflammatory skin disorder in which there are enlarging ulcers with undermined borders. The condition may occur in association with systemic diseases such as Crohn's disease and polyarthritis and can also develop after surgery.[1] Histologically there is massive infiltration with neutrophil granulocytes (PMN).[1] Treatments have included high doses of systemic corticosteroids, sulphonamide drugs including dapsone as well as clofazimine. Immunosuppressive drugs such as 6-mercaptopurine and azathioprine have also been used with varying success.[1]

In 1985, Curley *et al.*[2] reported the successful treatment of PG with oral cyclosporin A (CyA) in a dosage of 10 mg/kg daily. The improvement of the skin lesions was seen after 3 weeks of therapy and complete healing was achieved after a year of treatment with the dosage reduced to 4 mg/kg daily. These results were confirmed by further studies.[3] Recently, a total of eight patients with PG were treated with oral CyA in dosages of 8–10 mg/kg per day, and only one did not respond to treatment.[4,5] There was complete healing of the lesions in five of the patients.

We report a 51-year-old female patient who developed a lesion of PG on the left shoulder following surgery for a ruptured epithelial cyst. Conventional topical treatments that included corticosteroids over a period of 1 year had been unsatisfactory, and following reports[6,7] that intralesional CyA led to a complete remission of psoriatic plaques, we gave intralesional injections of a 1 : 3 dilution of Sandimmun® made up in isotonic saline to the active edges as well as beneath the lesion on two occasions in 1 week. The area of the lesion was about 20 cm^2 and the total dose of CyA injection was 35 mg. Two weeks after the injections there was improvement of the ulcer and complete healing occurred in 3 months. A small new lesion occurred at the margin of the healed ulcer 5 months after the first injection, but this disappeared following a further intralesional injection of CyA.

Apart from the pain from the intralesional injections that lasted for about 6 h, no other adverse effects were noted.

To our knowledge, this is the first report of the beneficial effect of intralesional CyA for the treatment of pyoderma gangrenosum. We used a lower dosage than described for the clearing of psoriatic plaques[6] and in further trials a lower dosage may also be effective. In previous reports[3,8,9] dosages of CyA > 5 mg/kg per day have been given systemically for the treatment of PG and have led to a number of drug-related side-effects. Thus the intralesional administration of CyA is preferable to systemic therapy. In psoriasis, CyA has proved to be an effective treatment when given systemically as well as by intralesional injection into the plaque.[6,7,10] Although the mode of action of CyA in both psoriasis and PG remains unknown, it is likely to be similar in both diseases.

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References

1 Wolff K, Stingl G. Pyoderma gangrenosum In: *Dermatology in General Medicine* (Fitzpatric TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds) New York. McGraw-Hill, 1987; 1328–36
2 Curley RK, Macfarlane AW, Vickers CFH. Pyoderma gangrenosum treated with cyclosporin A. *Br J Dermatol* 1985, 113: 601–4
3 Gupta AK, Ellis CN, Nickoloff BJ *et al.* Oral cyclosporine in the treatment of inflammatory and noninflammatory dermatoses. *Arch Dermatol* 1990, 126: 339–50.
4 Elgart G, Stover P, Larson K *et al.* Treatment of pyoderma gangrenosum with cyclosporine: results in seven patients. *J Am Acad Dermatol* 1991, 24: 83–6.
5 O'Donnell B, Powell FC, Cyclosporine treatment of pyoderma gangrenosum. *J Am Acad Dermatol* 1991, 24: 141–3.
6 Powles AV, Baker BS, McFadden J *et al.* Intralesional injection of cyclosporin in psoriasis *Lancet* 1988; i: 537.
7 Ho VC, Griffiths CEM, Ellis CN *et al.* Intralesional cyclosporine in the treatment of psoriasis. *J Am Acad Dermatol* 1990; 22: 94–100
8 Magid ML, Gold MH Treatment of recalcitrant pyoderma gangrenosum with cyclosporine. *J Am Acad Dermatol* 1989, 20: 293–4.
9 Elgart G, Larson K, Stover P, Davis B. Effective treatment of pyoderma gangrenosum with cyclosporin: a report of five cases. *J Invest Dermatol* 1989, 92: 424
10 Bos JD, Van Joost Th, Powles AV *et al* Use of cyclosporin in psoriasis. *Lancet* 1989, ii: 1500–2.

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Book Reviews

Retinoids: 10 years on. Edited by J.-H.Saurat (1991). Pp. 356. Basel: Karger. ISBN 3-8055-5387-0. Price £145.70.

The proliferation of papers published on retinoid research continues unabated, but it is somewhat surprising that a comprehensive text of recent discoveries has not been forthcoming. This compilation of presentations from the retinoid symposium 'Retinoids: 10 Years On', which was held in Geneva in November 1990, certainly goes some way to filling this gap. The book is divided into five sections: Advances in the biology of natural retinoids; Synthetic systemic retinoids: new fundamental aspects; Topical retinoids: present and future; Snythetic retinoids: scientific basis for clinical use; and Side effects. Many publications of proceedings of meetings are a collection of disjointed short papers, but this book provides us with excellent review-type articles. The recent discovery of nuclear retinoic acid receptors has transformed our understanding of how retinoids mediate their effects, but at the same time poses the question of what the functions of cellular retinoic acid binding proteins are. There are two articles devoted to nuclear retinoic-acid receptors, and they provide us with an excellent overview of their current status, and an updated reference list, in addition to papers on retinoid effects on epidermal differentiation and changes within the dermis. This volume also contains assessments of the clinical and therapeutic values of retinoids, and their side-effects.

As the topics covered are wide ranging, this book should appeal to both basic scientists and dermatologists alike, and I would strongly recommend it, although the price is likely to deter individual purchasers.

M.Edward

Advances in Dermatology, Volume 6. Edited by J.P.Callen, M.V.Dahl, L.E.Golitz, H.T.Greenway, Jr. and L.A.Schachner (1991) St. Louis: Mosby Year Book. ISBN 0-8151-1419-2. Pp. 346 Price £42.50.

This is the sixth volume in a series which covers a wide range of dermatological topics. This issue is divided into sections on internal medicine—therapeutics and skin disease, dermatologic surgery, paediatric skin disease, clinical research, and dermatopathology. In the first section the appraisal of the so-called dysplastic naevus syndrome by Piepkorn is one of the most balanced accounts of what can sometimes be an emotional topic that I have yet read. The section on dermatologic surgery includes a rather advanced chapter on foot and ankle surgery and a useful account of lasers in 1990. In the paediatric section I personally found the review of cutaneous immunodeficiency disorders particularly helpful and in the section on dermatopathology was impressed by the comprehensiveness of Uitto's review on molecular pathology of collagen in cutaneous diseases. One of the original ideas in this series is an editor's comment at the end of each section. Perhaps it is inevitable that these are rather bland and I do wonder if they really serve a useful purpose. The book is recommended and is modestly priced by today's standards.

Rona M.MacKie

British Journal of Dermatology (1991) 125, 501.

News and Notices

Introductory course on the Biology of the Skin, Cambridge, 9–13 December 1991

The annual introductory course 'The Biology of the Skin' will be held in Fitzwilliam College, Cambridge, 9–13 December 1991. This course is primarily for registrars and postgraduate students at an early stage of their training in dermatology but is open to other interested practitioners. Closing date for applications 18 November 1991. Further information may be obtained from: the Conference Secretary (BOS), Postgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Telephone 0223-212216.

European Society for Dermatological Research 4–7 April 1992, London

The 22nd Annual Scientific Meeting of the ESDR will be held at Kensington Town Hall, London on 4–7 April 1992. For further information contact: Dr D. M. MacDonald, President ESDR, Dermatology Department, Guy's Hospital, St Thomas Street, London SE1 9RT. Telephone: 071-955-4584. Fax: 071-357-7563. The deadline for abstracts will be 15 December 1991.

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There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g
kilogram(s)	kg
milligram(s) (10^{-3}g)	mg
microgram(s) (10^{-6}g)	μg
nanogram(s) (10^{-9}g)	ng
picogram(s) (10^{-12}g)	pg
hours(s)	h
minute(s)	min
centimetre(s)	cm
second(s)	s
cubic millimetre(s)	mm^3
millimetre(s)	mm
millicurie(s)	mCi
milliequivalent	mmol
molar	mol l^{-1}
millilitre(s)	ml
gravitational acceleration	g
micrometre(s)	μm
per cent	%
isotopic mass number placed as	^{131}I

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations. A Guide for Biological and Medical Editors and Authors* (1988) The Royal Society of Medicine, London.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be given in the Vancouver style, i.e. references appear as superscript numbers in the text, e.g. '. . . our previous report[1] and that of Smith *et al.*[2] . . .', and are brought together numerically in the reference list at the end of the article in order of their first mention in the text. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s), up to four authors; if more than four authors, include first three authors followed by *et al.*; (2) title of paper; (3) title of journal abbreviated in the standard manner; (4) year of publication; (5) volume; (6) page numbers of the article. *Thus*: Cunliffe WJ, Shuster S. The rate of sebum excretion in man. *Br J Dermatol* 1969; 81: 697–9. References to books and monographs should include: (1) author(s) or editors(s); (2) paper (if necessary) and book titles; (3) edition, volume, etc.; (4) place; (5) publisher; (6) year; (7) page(s) referred to. *Thus*: Beare JM, Wilson Jones E. Necrobiotic disorders. In *Textbook of Dermatology* (Rook AJ, Wilkinson DS, Ebling FJ, eds), 2nd edn., Vol. 2. Oxford: Blackwell Scientific Publications, 1972; 1066.

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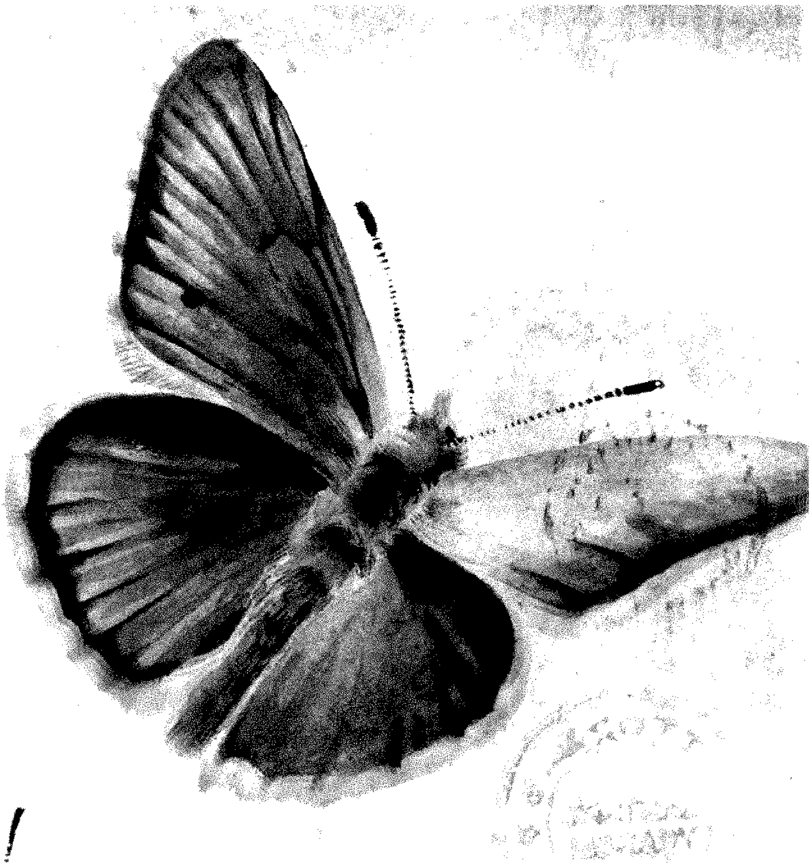
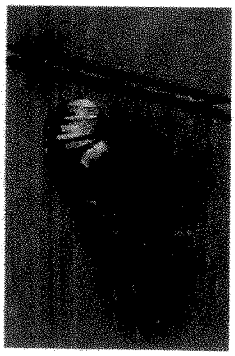
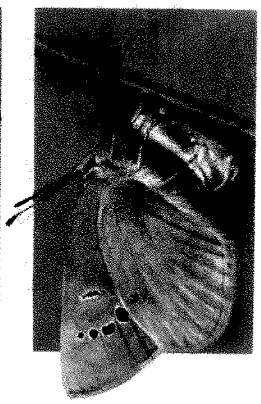
A totally new treatment emerges in psoriasis







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- effective
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Dovonex is an unique topical treatment that i
odourless, non-staining and avoids the adve
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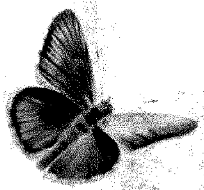
Dovonex

▼ calcipotriol



Prescribing Information. Indication: Mild to moderate plaque psoriasis affecting up to 40% of skin area. **Presentation:** A 30g tube of Dovonex ointment, 50 micrograms calcipotriol per g in a smooth, white, preservative-free base. **Dosage and Administration:** Apply twice daily to the affected area. A course of treatment should not last longer than 6 weeks and the weekly dose should not exceed 100g. No experience in children. **Contra-Indications:** Patients with known calcium metabolism disorders. Hypersensitivity to any constituents. **Precautions:** Should not be used on the face. Wash hands after applying the ointment. **Drug Interactions:** No experience of concomitant therapy with other topical antipsoriatic products. **Side-e** Transient local irritation. Facial or perioral dermatitis. **Basic N.H.S. Price: Product Licence Number:** 0043/0177.

L E O

Further information available on request from
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